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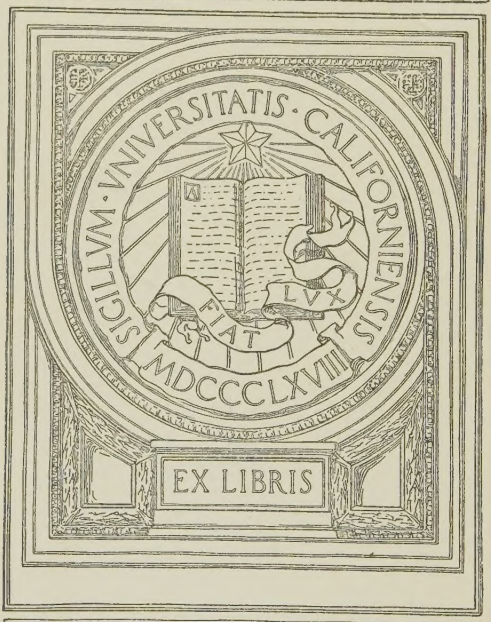

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RECENT ADVANCES IN MEDICINE

CLINICAL LABORATORY THERAPEUTIC

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FIFTH EDITION

WITH 49 ILLUSTRATIONS

PHILADELPHIA

P. BLAKISTON'S SON & CO., INC.

1012 WALNUT STREET

1930

First Edition, 1924.
Second Edition, 1925.
Third Edition, 1926.
Fourth Edition, 1928.
Fifth Edition, 1929.

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PREFACE TO THE FIFTH EDITION

In this edition we have omitted certain sections in order to afford space for additions, and at the same time keep the book within reasonable limits.

The chapter dealing with the diagnosis of enterica infections, and the greater part of the chapter on disorders of the endocrine system have been left out. Read's formula has been incorporated in the account of basal metabolism, and the work on the parathyroids and calcium is included in the conclusions to the last chapter on the value of blood analysis. Certain laboratory methods, such as Sellard's test, the benzoic acid test for renal function, Sahli's test, Cammidge's reaction, the Noguchi and Pandy method and one variation of the Lange gold chloride test have also been omitted.

This has allowed space for new sections. The first deals with nephritis. A clinical classification of its types, together with the clinical and bio-chemical findings is given, and the treatment is discussed, various diet sheets being included. The modified Sippy treatment of gastric ulcer has been written at greater length and typical diet sheets given for the different stages of the disease. The account of the treatment of diabetic coma has been considerably amplified, and the important aspect of circulatory failure considered. The liver treatment of pernicious anæmia has been discussed in detail.

The methods for blood and urine analysis in the last chapter have been brought up to date. Two new figures are introduced, and the additions include the colorimetric methods for the estimation of blood proteins, hæmoglobin,

26961

the total solids in the blood, the icterus index, the estimation of protein in the urine and the albumin-globulin ratio.

Each chapter has been carefully revised, and alterations and additions made : amongst the latter may be mentioned Andrewes' reaction in uræmia, synthalin in diabetes, the approximate amounts of sugar in the urine as shown by the colour changes with Benedict's solution, and the amount of protein in the urine corresponding with the density of the turbidity obtained on boiling. A section has been written on the microscopical examination of fæces, and MacLean's work on lactic acid in the stomach contents is given ; the importance of porphyrins in the fæces is described in the occult blood test.

Dr. Langdon Brown has kindly given us permission to include his modified Lenhartz table, in place of the original one which is now of historic interest rather than of practical value. The chapters on the Schick and Dick re-action have been revised, and the methods and results of active immunisation included.

Such a revision has meant alterations in the arrangement of the book, and we are much indebted to our publishers for granting us every facility. Those methods have been included which we have found of real clinical value in the investigation and treatment of disease, and the requirements of practitioners for a detailed line of treatment have been borne in mind. In this way we hope to maintain the book as a record of those "Recent Advances in Medicine," which are of proved practical value.

G. E. B.
E. C. D.

London.

PREFACE TO THE FIRST EDITION

During the last decade changes have taken place in medicine, especially in the routine methods adopted in the clinical and laboratory investigation of disease, and also in certain forms of treatment.

This book has been compiled with the following objects : to assist practitioners who have not had the opportunity of recent post-graduate study, to familiarise themselves with some of these advances ; to provide a reference book for those who are working for the higher examinations in medicine ; to give candidates studying for the primary examination for the Fellowship of the Royal College of Surgeons an account of the application of physiological and biochemical principles to medicine. It is also hoped that it will prove of assistance to the laboratory worker in that the recent chemical methods are dealt with in detail. It should also form a link between the wards of a hospital and the laboratories, giving fuller details of methods which are alluded to in medical textbooks, but often omitted from the handbooks on clinical methods. The recent work of American and Continental authorities has also been incorporated.

One of the chief difficulties has been to decide the actual scope of the contents, which of necessity encroach upon the domains of theoretical medicine, therapeutics, biochemistry and bacteriology. The guiding principle has been to confine the subject-matter to a description of such methods of diagnosis and treatment as are used for medical patients in a general hospital, and which can be justly termed " recent advances in medicine."

Great care has been taken to give a workable description

of each procedure, all techniques described have been performed personally by one or other of us, and the accounts are taken from our notebooks. Although these may differ slightly from the original descriptions, the methods described have been used by us as a routine for some time, and have given very satisfactory results. We have attempted to state the value of the results obtained by the various tests.

The authors wish to express their indebtedness to the numerous writers whose works have been consulted, and an endeavour has been made to acknowledge them by the list of references.

We have pleasure in thanking Sir Thomas Lewis for permission to reproduce the electrocardiograms taken from his book entitled " Clinical Electrocardiography." Dr. D. E. Bedford has supplied the polygraph tracings, and has also read through the proofs of the chapter dealing with the heart. We are grateful for his valuable suggestions and help. We desire also to thank Dr. T. Izod Bennett for his criticism of the chapter on the stomach, and for permission to reproduce the charts illustrating the results of fractional gastric analysis which appeared in the *Journal of Clinical Research.* Further, we wish to express our thanks for the loan of certain blocks : to Messrs. Hawksley & Sons for the one illustrating the Jacquet polygraph ; to Messrs. Allen and Hanbury for the block of the Burrell aspirator ; to Messrs. Down Bros. for those illustrating the pneumothorax needles ; and to Professor Harris for the diagram of the electrocardiograph from Anrep and Harris' " Practical Physiology."

In conclusion we wish to acknowledge the unfailing help which we have received from the publishers of this volume.

G. E. BEAUMONT
E. C. DODDS

London, 1924

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RECENT ADVANCES IN MEDICINE

CHAPTER I

CLINICAL INVESTIGATIONS

Despite a careful clinical examination, the importance of which cannot be over-estimated, it is often impossible to arrive at a correct diagnosis in medical cases without employing further investigations based on certain recent advances in medicine. Patients complain of symptoms, or on examination signs are found, referable to certain parts of the body or systems, and various modern methods are of value in their elucidation.

An outline of the clinical application of such methods may aid the practitioner in his selection of the investigations required in particular cases, and also indicate what benefit the patient may expect to derive from allowing them to be performed. In many instances where the diagnosis of the disease is in doubt it may be necessary for the patient to be admitted to a clinic, hospital or nursing home for further examinations. In other cases special forms of treatment are required which can only be adequately and safely initiated in institutions equipped with laboratory and X-ray facilities.

The indications for the scope of such investigations, the nature of cases in which they are required, and the help that may reasonably be expected to accrue from them will be suggested in this chapter. The actual details of the

examinations are described in the subsequent portions of the book, and those diseases alone will be considered here to which these methods are applicable. For convenience the conditions which may require investigation may be classified according to the system of the body principally affected.

Digestive Disorders.—A large proportion of cases requiring special investigation are those where "indigestion" of one sort or another is the prominent feature. In the majority of such cases a routine medical examination is apt to be inconclusive unless there are definite signs of a tumour present in the abdomen, or of the digestive symptoms being secondary to disease of some other part of the body, such as of the heart, lungs, or central nervous system. In the primary digestive disorders, therefore, the diagnosis is usually arrived at largely by a careful consideration of the history and the symptoms of the case. In order to establish the diagnosis, however, the practitioner requires more accurate information, especially with regard to the secretion of the stomach, whether the acidity is greater or less than normal, the rate of stomach emptying and its position in the abdomen, whether or not there is any indication of gastric or duodenal ulcer, or of gastric carcinoma. It may also be necessary to determine the presence or absence of cholecystitis or of gall-stones, and examinations may be required to see whether there is any deficiency on the part of the pancreas or liver. The rate of passage of the food through the stomach or intestines, and the appearance of the appendix, if shown by an opaque meal, will likewise throw valuable light upon symptoms in certain cases.

For a complete investigation of the gastric function, a test meal is required, and the information yielded by the fractional method of gastric analysis is certainly more instructive than that afforded by the one-hour method. By this means the rate of stomach emptying, the presence or absence of gastric stasis, the acidity of the gastric juice, and at times the indications of a new growth in the stomach may be ascer-

tained. In such cases also the fæces should be examined for the presence of occult blood, for if an obvious source of hæmorrhage, such as the presence of piles, be excluded, and if the test be carried out with due precautions as to diet, the finding of blood in minute quantities is valuable evidence of an organic lesion in the alimentary tract. The X-ray examination, after administration of an opaque meal, will show the size and position of the stomach, the presence of persistent deformity resulting from an organic cause such as stricture or contraction, the rate of gastric emptying and in some cases deformity due to the presence of an ulcer crater. Irregularities of the first part of the duodenum, called from its shape the duodenal cap, are more difficult to interpret, as they may be caused by a lesion such as an ulcer, by a reflex contraction of its wall, or by pressure from without due to an enlarged gall-bladder, glands in the portal fissure or other causes. The rate of passage of the meal through the intestines can also be gauged, visceroptosis can be visualised, and in some instances signs of disease of the appendix or the appearance resulting from diverticulitis of the intestines can be seen. The methods for carrying out these investigations and the treatment of gastric and duodenal ulcers are described in Chapter VI.

Pancreatic Disorders.—Chronic symptoms of indigestion are more rarely due to lesions of the pancreas. Certain symptoms, however, may indicate the possibility of such an origin, and justify further tests being performed. Thus if patients complain of wasting, loss of appetite, abdominal pains and the passage of bulky and perhaps oily stools, a suspicion of a pancreatic origin for their troubles would be justified. Other symptoms such as glycosuria or jaundice, might also warrant an investigation of the pancreatic function. The methods to be adopted in such cases are described in Chapter IV.

It is usually difficult to differentiate clinically acute pancreatic lesions from other acute abdominal catastrophies. Examination of the urine, to determine its diastase content,

is a test of very great value, as in acute pancreatitis the diastase figure is considerably raised (see Chapter IV.).

Hepatic Disorders. —An accurate clinical diagnosis of chronic cholecystitis, especially when occurring in patients who have never suffered from an attack of biliary colic or jaundice, presents great difficulties. The symptoms of indigestion in such cases are often suggestive, but are admittedly inconclusive ; further investigations are thus of value, and may be carried out on the following lines: The blood is examined for the presence of bilirubin in excessive amount, either by the Fouchet or van den Bergh tests (see Chapter V.). A sample of bile may be withdrawn through a duodenal tube after the gall-bladder has been stimulated to contract by the administration of magnesium sulphate, in accordance with the Lyon technique. The results of this test, however, have been very disappointing. An X-ray photograph may be taken to see if gall-stones are revealed. A more recent test is that of cholecystography, which is also described in Chapter V. In this process, a drug such as tetraiodophenolphthalein is administered by mouth or intravenously ; after absorption it is carried by the blood to the liver and excreted in the bile. As it is opaque to the rays, it reveals the outline of the gall-bladder, and the smooth and regular contour of the normal bladder can be distinguished from that deformed by the presence of stones or distorted by inflammatory changes or adhesions in or around its walls. The limitations of this test are discussed in Chapter V.

The practitioner is often faced with conditions in which he believes the hepatic or biliary functions are at fault. In clinical states of jaundice, the van den Bergh test is a means of differentiating between an obstructive or non-obstructive cause. The technique and limitations of this procedure are described in Chapter V.

The actual tests for hepatic function, although very numerous, are on the whole somewhat disappointing. In certain cases they may, however, yield useful information.

Thus if eclampsia be threatening, the hepatic and renal function tests, considered in conjunction, are helpful guides to treatment. They have also been used during the course of salvarsan therapy, if an idiosyncrasy to the drug be suspected, and may then indicate whether or not it is dangerous to proceed with the treatment.

Renal Disorders.—One of the greatest difficulties with which a practitioner may be faced is to decide whether a patient who has albuminuria is suffering from a condition due to, or likely to lead to permanent renal damage. In such cases, in addition to the clinical examination of the heart, arteries, blood pressure and optic discs, and observations on the relation of the albuminuria to posture or exercise, special examinations for renal function should be carried out. These are considered in Chapter II.

The importance of a chemical examination of the blood should not be overlooked ; the estimation of the urea and non-protein nitrogen is of value especially in cases of chronic nephritis without œdema, whereas the blood cholesterol is most likely to be raised in chronic nephritis with œdema. The prognosis is more unfavourable if the amount of these substances in the blood is found to be raised, than if they remain at their normal level. The blood cholesterol, protein content and albumin-globulin ratio, are also of value in indicating whether a high or low protein diet should be given. Estimation of the blood calcium and phosphorus will give a valuable indication as to prognosis, since a fall in the former and a rise in the latter constitute a grave sign. There are also several tests of renal function which do not require elaborate apparatus and can be carried out by the clinician, such as observations on water excretion, renal test meals, and the urea concentration test. These will give a rough idea as to the excreting power of the kidney.

In the albuminuria of pregnancy various clinical problems arise, the solution of which is discussed on pp. 30–32. In all cases of projected operation upon the prostate gland,

blood examinations are a necessary preliminary in order to determine whether or not the patient is fit to stand the operation. The blood urea and non-protein nitrogen content should be determined, and the significance of the findings is discussed at the end of the chapter on renal function. The reasons why such blood examinations have entirely superseded the older clinical method of estimating the urea in the urine are given on p. 23. In coma, due to suspected uræmia, in addition to observations on the urine, chemical examinations of the blood and cerebro-spinal fluid are of great value, as the urea and non-protein nitrogen figures are usually although not invariably raised in both these fluids. The absence of blood from the cerebro-spinal fluid is also evidence against the coma being due to a cerebral hæmorrhage which is so liable to occur in chronic renal disease.

An account will also be found in this chapter of the treatment of acute, subacute and chronic nephritis and of uræmia.

Respiratory Disorders.—Patients who suffer from hay fever or asthma may have their cutaneous protein reactions tested. In hay fever this is chiefly of importance in order to determine during the winter whether or not the patient is in reality sensitive to pollen, and if so to desensitise him before the ensuing hay fever season begins. The prophylactic method of treatment is described in Chapter X. In asthma the cutaneous tests should also be performed, as in some instances in which the patient is found to be sensitive to proteins, a cure may be effected by removing the offending substance. This is especially so when the patient is found to be susceptible to feathers, which are usually present in pillows, cushions or eiderdowns. In other cases, however, it may be impossible to remove the protein from the patient's environment, as for example, in asthma due to horse dandruff, or to cats and dogs. In such instances an attempt at desensitisation should be made by repeated injections of small doses of the protein. In other cases the asthma appears to be more complex, as for example, when a patient gives a positive skin

reaction to some article of food such as fish, but removal of the substance from the dietary does not effect a cure. The methods for carrying out these investigations may be found in Chapter X.

In obscure diseases of the chest, as when a mediastinal or pulmonary new growth or cyst is suspected, and when the X-ray appearances are inconclusive, the establishment of an artificial pneumothorax may enable a clear and more decisive radiogram to be obtained. This is especially so if the underlying condition is obscured by a pleural effusion. A gas replacement of the fluid is then of the greatest value, as after the effusion has been aspirated and replaced by air, a clear view of the condition of the pulmonary root can be obtained by X-rays. In pulmonary tuberculosis, bronchiectasis and abscess of the lung, the induction of an artificial pneumothorax in many instances constitutes an important advance in treatment, and a decision should always be made as to whether or not it is indicated. Such considerations and the technique of the operation are described subsequently in Chapter IX. In many instances in which the patient complains of expectoration of offensive sputum, it is difficult to decide by ordinary clinical or radiological examination whether a bronchiectatic cavity is present. An X-ray examination after the injection of lipiodol into the trachea is of value in revealing dilatation of the bronchi, and also shows the extent of lung involved, and whether the disease is bilateral. The information thus obtained is essential in deciding the nature of treatment to be adopted, as described in Chapter IX. The ordinary medical methods of treatment of bronchiectasis, such as the use of creosote by the mouth or inhalation, the administration of vaccines, and postural attempts at drainage have in our experience proved practically valueless. The more recent procedures such as artificial pneumothorax, or if this be impossible owing to the presence of pleural adhesions, surgical measures such as evulsion of the phrenic nerve, or thoracoplasty constitute an important therapeutic ad-

vance, the benefit of which should be offered to the sufferer from such a disease. In the case of pleural effusions it is at times advisable to aspirate the fluid and replace it by air ; the reasons for doing so and the technique of the operation are described in Chapter IX. These special investigations are best carried out in a hospital or fully-equipped clinic.

Metabolic Disorders.—In certain metabolic diseases it is essential that special investigations should be performed in order to determine the diagnosis and correct line of treatment. Thus if an examination of the urine show that sugar is present, the sugar tolerance should be estimated by means of blood sugar readings after administering by mouth a dose of glucose. In this way it is possible to say whether the patient is suffering from diabetes mellitus or from a more innocent condition, such as renal glycosuria. These methods are described in Chapter III. The modern treatment of diabetes, both with and without the administration of insulin and also the treatment of coma, is detailed in this chapter.

Acidosis may occur in conditions apart from diabetes ; thus not infrequently young children suffer from feverish attacks with gastro-intestinal symptoms due to a metabolic disturbance. The urine in such cases should be tested by Rothera's method for the presence of ketone bodies, and if found in large quantities a reduction of the amount of fat in the diet and the administration of sodium bicarbonate and glucose by the mouth will usually be followed by a rapid disappearance of the symptoms. Examination of the blood is helpful in the diagnosis of gout. The uric acid may be found to be definitely raised in patients of a gouty diathesis, but a significant increase is only to be found during the acute stages of the disease. The method of performing this investigation is described in Chapter XV.

Diseases of the Ductless Glands.—The elucidation of the nature of disturbances of internal secretion is often a matter of great difficulty. Considerable help can be obtained by the various methods described later. Thus the estimation of the

basal metabolic rate serves to differentiate between conditions due to over or under activity of the thyroid gland. A good guide to the basal metabolism can be obtained in such cases by using Read's formula in which the only data required are the pulse rate and pulse pressure. Basal metabolic readings are also of value in the treatment of Graves' disease, as a guide to the advisability of surgical intervention. These methods are described in Chapter VII. Further investigations which are required in order to appreciate the degree of metabolic disturbance or of toxæmia resulting from hyperthyroidism are observations upon the sugar tolerance and an electrocardiogram.

Cardio-vascular Diseases.—One of the greatest advances which has been made during recent times in the field of clinical medicine is the explanation of the nature of cardiac irregularities. In every case in which the pulse is found to be irregular the physician should endeavour to decide what is the type of the irregularity, for without such knowledge its true significance cannot be appreciated. In many cases this can be done without the use of special instruments, and in cases of this kind it is usually possible to recognise clinically such disturbances of cardiac rhythm as sinus arrhythmia, premature systoles, certain grades of heart-block and auricular fibrillation. The characteristic features of these irregularities, together with the indication for the use of special instruments and the results obtained thereby, are discussed in Chapter VIII.

From the therapeutic aspect, digitalis is undoubtedly the drug of greatest utility in heart failure with abnormal rhythm, but in order to obtain the best results it must be prescribed in accordance with certain fairly well defined rules. Quinidine is also a powerful remedy, but its administration is not without danger, and the principles underlying the therapeutic uses of these drugs are discussed in the same chapter.

Blood pressure estimations should figure in the routine clinical examination of every patient, and observations on

the systolic and diastolic readings often throw light upon obscure cases. If the blood pressure be high and there be no apparent cardio-vascular or renal lesion, a chemical examination of the blood should be made to ascertain whether there is nitrogen retention. Nitrogen retention may be due to intestinal toxæmia and yield to measures directed to the relief of such a condition, or it may be an early manifestation of renal damage.

Diseases of the Nervous System.—In spinal cord compression changes are found in the cerebro-spinal fluid which are known as the "loculation syndrome." An intrathecal injection of lipiodol, either by cisternal or lumbar puncture, is, in some instances, of value in localising the site of the obstruction. These methods are described in Chapter XIII. Examination of the cerebro-spinal fluid is essential for the diagnosis of many nervous diseases. This applies particularly to cases of meningitis or syphilitic affections of the central nervous system. The cerebro-spinal fluid in such diseases undergoes remarkable, and in many cases specific alterations which are described in this chapter, together with the methods of performing the tests. In cases of coma a lumbar puncture is also of great value, for in this way such conditions as cerebral hæmorrhage, uræmia and diabetes can often be diagnosed. In the early stages of tabes dorsalis the physical signs are often slight and indefinite, and the cerebro-spinal fluid must be examined before a definite diagnosis can be made. Thus, repeated attacks of abdominal pain may be ascribed to a gastric or duodenal lesion whereas, in reality, they are manifestations of a tabetic crisis.

Blood Diseases.—The diagnosis in these cases is usually established by means of blood counts and hæmoglobin estimations. In some instances, special investigations, which are described in later chapters of the book, are of additional value. Thus, in pernicious anæmia it is found that even in the period of remission of the disease the average size of the red cells is greater than normal. Their diameters can be

measured and graphically recorded in the form of the Price-Jones curve. In this disease two other investigations are useful as confirmatory evidence; thus, there is almost invariably an increase in the bilirubin content of the blood, which gives an indirect van den Bergh reaction, and examination of the gastric contents after a test meal shows an absence of free hydrochloric acid. These special investigations are not necessary in clear-cut and typical cases of pernicious anæmia, but not infrequently somewhat indefinite cases of a severe anæmia are encountered in which the colour index is just above unity, and yet the other blood changes are inconclusive. It is particularly in this type of case that these additional aids to diagnosis are of value.

In acholuric jaundice the chief clinical signs are a slight degree of icterus of the skin and conjunctivæ with enlargement of the spleen. An estimation of the fragility of the red corpuscles serves to differentiate acholuric jaundice from other diseases such as splenic anæmia, which are apt to cause difficulty. In the former the fragility of the red cells is definitely increased, which is not observed to such an extent in any other condition. The treatment of pernicious anæmia by the administration of liver is also described in Chapter XIV.

Infectious Diseases.—The recent advances which have been made in certain diseases of this group are included in Chapters XI. and XII. In diphtheria the Schick reaction enables the doctor to decide whether the contacts of a case or other members of a family or school are susceptible. In this way, individuals who are found immune can be spared the restriction of isolation during the quarantine period. A method of desensitisation is also described which, by actively immunising those found susceptible to diphtheria, will minimise the risk of infection and spread of disease.

A somewhat similar test, the Dick reaction, is described for scarlet fever. This is chiefly of value in preventing the spread of infection, by determining which of the contacts of a

case of scarlet fever are susceptible. The methods of desensitisation by active immunisation against scarlet fever are not so valuable at present as in diphtheria. The use of a scarlet fever antitoxin for the treatment of the disease is also described.

CHAPTER II

TESTS OF RENAL FUNCTION

The tests of renal function are very numerous, but they fall naturally under the following headings :—

1. The presence of abnormal urinary constituents, such as protein, casts, etc.

2. The altered physiological balance between blood and urine. This depends upon analysis of the blood or urine, or both.

3. The eliminating power of the kidney as tested by the administration of :—

(*a*) Some natural substances, such as—

(i.) Water.

(ii.) Urea.

(iii.) Renal test meals.

or

(*b*) Some foreign substance, such as—

A dye, as phenol sulphone phthalein or indigo carmine.

The choice of these methods depends very largely on the fancy of the investigator. All employ group (i.) as a routine, and adopt one or more of the remaining tests according to their experience. It is impossible for an observer to recommend any one test and condemn the others, since all give more or less valuable information, and it is unlikely that any one person has had a sufficiently wide experience of all the tests. In the present account we will therefore describe tests which are held by various authorities to yield reliable information, and will indicate those which, in our experience, have given the best results.

At the outset, it must be pointed out that the investigation of a patient suffering from renal disease in hospital is quite a different problem from the investigation of a private patient at home. Apart from the question of time, apparatus and skill it is well known that it is all but impossible to get an accurate twenty-four hours' specimen of urine unless the patient is under strict supervision, hence any test depending on this will naturally be unsuited to the needs of the practitioner. We have found that blood analysis is amongst the most suitable for this purpose, since all that is needed is the collection of a sample of blood, and its despatch to a laboratory, which will furnish a report in a short time. The blood findings, taken in conjunction with clinical observations, are of great value. Again, the type of test selected depends on whether the case is a surgical or a medical one. It will, perhaps, be well to describe the techniques, and summarise their relative advantages and disadvantages in conclusion.

GROUP I

Tests Depending on the Presence of some Abnormal Constituent in the Urine

Proteins.—*Albumin.*—This is usually detected in the routine analysis of urine as soon as a case comes under observation.

Tests.—*The Boiling Test.*—This is by far the most delicate and the easiest to perform. It consists in boiling the top of a column of urine in a test tube. The urine must be clear, and, if necessary, filtered. A drop or so of 10% acetic acid must be added, since urine verging on neutrality will precipitate phosphates owing to a lowering of the acidity due to boiling off CO_2. If albumin be present a cloud appears at the top, and is easily distinguished by contrast with the clear unboiled layer below. As the delicacy of the test depends on this contrast, the necessity for having the urine clear at the start is obvious. The only fallacy in this test

is the presence of Bence-Jones proteose. This body will be detected by the fact that it appears at about 50° C., but disappears on boiling and reappears on cooling.

The Salicylsulphonic Acid Test.—This reaction, though not so delicate as the boiling test, is particularly useful when only small quantities of urine are available. The technique consists in taking an equal column (1 c.c. or so) of urine in two test tubes, and adding 2 or 3 drops of saturated salicylsulphonic acid solution in water to one of them. If albumin be present, the liquid in the tube containing the reagent will appear turbid on comparison with the control. Again, the delicacy of the reaction consists in the comparison of the urine tested, with the original urine.

Globulin.—The association of globulin with albumin in the urine has been known for a very long time.

Tests.—1. The appearance of a precipitate in the cold on the addition of dilute acetic acid to the urine.

2. The production of " rings " on dropping urine into a tall glass vessel containing distilled water.

3. Precipitation by saturation with magnesium sulphate.

In the past great stress has been laid on the relative amounts of albumin and globulin present in the urine. This was determined by Noël Paton's application of Esbach's method (1), but the results proved far from satisfactory, and differentiation of cases by this means was abandoned. Recently, however, the whole question of the quantitative estimation of protein in the urine has undergone a striking alteration.

Hiller, McIntosh and Van Slyke (2) have described a very satisfactory means of estimating protein quantitatively by an application of Autenrieth's colorimetric biuret method. In the technique worked out by them the colour given by the proteins with copper sulphate and alkali is compared with that given by a standard solution of biuret treated in the same manner, and from this the protein content can be worked out. This gives the total protein, and by adopting a salting-out procedure, it is possible to estimate the albumin-

globulin ratio. The details of this technique are described under "Urine Analysis" (p. 413), whilst the application of this method to the analysis of blood for proteins is described on p. 395.

The authors quoted above state that the albumin-globulin ratio is usually found to be above 10 in cases of nephrosis, and between 5 and 10 in acute nephritis. The ratio is low during the early stages of acute nephritis and it rises as recovery takes place. If it be below 5 an advanced state of chronic glomerulo-nephritis with urea retention and impairment of renal function is generally found. They record one case of amyloid nephrosis with a very low ratio, namely 1·5. "The low ratios in advanced chronic nephritis were associated with low urea concentration indices and poor prognoses. Of fifteen cases observed with average urinary albumin-globulin ratios below 5, all but two have died in less than eighteen months, and these two have shown progressive decrease in renal function."

It is interesting to note the relation between the boiling test for albumin and the exact quantities of protein present in the urine as determined by the above method. Lawson (unpublished results) found that a just perceptible trace by the boiling test corresponds to 0·1 g. per litre, whilst a distinct cloud, easily recognisable, corresponds to 0·5 g. per litre; a heavy cloud is given by urine containing 2·0 g. per litre or over. He found that it was very important to note how much acetic acid was added to the urine prior to boiling. The test appears to be at its greatest delicacy if the urine be boiled first in the upper part of the test-tube, and then 2 drops of 10% acetic acid be added after boiling.

Casts.—The urine should be centrifugated and the deposit examined for casts.

The presence of casts must always be regarded as a serious sign, the gravity of which depends upon the type present. Casts are usually classified as follows:—

1. *Granular casts* are composed of a mixture of albumin,

leucocytes, and red blood corpuscles. They are present usually in chronic nephritis.

2. *Hyaline casts* are translucent elongated bodies, common to all nephritic conditions, and are very difficult to detect.

3. *Epithelial casts* are composed of coagulated albumin, and bear the epithelial cells of the tubules on their surface. They are very numerous in acute nephritis and their presence means that the kidney is being seriously and probably permanently damaged.

4. *Blood casts* are found in any condition in which there is bleeding from the organ. Thus they are present in acute nephritis, congestion, infarct, etc.

5. *Fatty and waxy casts* appear in chronic renal disease, and the latter particularly in amyloid disease.

6. *Pus casts* only appear when there is actual suppuration in the kidney.

Illustrations of these deposits are omitted, since they are so frequently depicted in text books. The identification of urinary deposits is a very difficult matter, and can only be learned by examining actual specimens under the direction of an expert.

Blood.—This may appear in the urine in nephritis, and thus constitute one of the abnormal constituents of urine, but in systematic and routine examinations albumin and casts are by far the most important. Blood is best identified by examination of a centrifugated deposit, or by the guaiacum test (see p. 181).

Pus and Mucin.—The presence of pus in the urine can be determined by staining a smear of the deposit obtained by centrifugation. The presence of polymorphonuclear leucocytes in large numbers indicates the presence of pus. Very little reliance can be placed on such tests as the liquor potassæ reaction. Mucin is a normal constituent of the urine, and may often be seen in the form of a faint cloud. It may be present in pathological conditions in excess. The

addition of acetic acid in such cases will cause a precipitate, which is insoluble in excess of acid, but is soluble in dilute alkali. To demonstrate the presence of nucleo-proteins and mucin in the presence of albumin and globulin, the following test is recommended. The urine is boiled and filtered to remove albumin and globulin. Ten c.c. are then diluted to 40 c.c. with water, and the reaction is made strongly acid with acetic acid. A turbidity indicates the presence of mucin or nucleo-proteins. The urine must be diluted as described, otherwise a precipitate of urates will appear.

GROUP II

Tests Depending on the Altered Physiological Balance Between the Blood and Urine

With a knowledge of the functions of the kidney, and of the physiological composition of blood and urine, it should be possible to detect renal lesions by an upset in the balance between the blood and urine.

There are three possible ways of attacking the problem from this point of view :—

By blood analysis alone.
By urine analysis alone.
By a combination of both.

It will be well to consider these separately.

Renal Function as Tested by Blood Analysis

Owing to the perfection of methods of blood analysis, and to the relative simplicity of technique, this type of renal function test has been used extensively. Analysis of the blood, as a guide to diagnosis and prognosis, is perhaps the most useful method both in hospital and general practice.

In the former, analyses can be done on large numbers of cases, and statistical figures can be worked out, whilst in general practice this method is particularly useful, since it

relieves the practitioner of all responsibility other than that of collecting the blood. Ten or twenty c.c. are collected by vein puncture in the manner described later (p. 373), and are preserved in an "oxalate" or "citrate" tube, which is despatched to the laboratory for analysis.

Urea and non-protein nitrogen contents are held by most authorities to yield the most reliable information, and with this we are in complete agreement. Before proceeding to the interpretation of the results of analysis, it will be necessary to summarise briefly the current physiological views on these subjects. It will be remembered that proteins are digested to amino-acids in the alimentary canal, from which they are absorbed into the blood stream to undergo exogenous and endogenous metabolism.

The end product of the former is urea, and of the latter various substances, such as creatinin and uric acid, all of which are excreted by the kidney in such a manner as to keep the concentration more or less constant in the blood.

Analyses of blood obtained from normal people have been performed by many workers, such as Folin and Wu (3), and the following can be taken as fairly typical of the existing opinions on normal blood :—

Urea . . .	20 to 40	mg. per 100 c.c.
Non-protein nitrogen .	20 to 40	,, ,,
Creatinin . . .	1 to 1·5	,, ,,
Uric acid . . .	2 to 3·5	,, ,,
Cholesterol . .	180 to 225	,, ,,
Chlorides . . .	500	,, ,,

In certain forms of nephritis, *i.e.*, the nitrogen retention or azotæmic type, the kidney fails to excrete these bodies efficiently, with the result that their concentration is increased. Thus a blood urea, or non-protein nitrogen content of over 40 mg. per 100 c.c. would indicate impairment of renal function, provided it could be established that renal disease alone can cause an increase in these figures. Unfortunately, certain other conditions, such

as prolonged vomiting, intestinal obstruction, and acute abdominal lesions may cause an increase in the constituents of the non-protein nitrogen. If there be any likelihood of such a condition, it is advisable to estimate the urea in a specimen of urine withdrawn at the same time as the blood. If the nitrogen retention be due to renal disease, the urea content will be below 2%, whereas with non-nephritic causes, the urea concentration will most probably be 2% or over. Despite what is said to the contrary, the nitrogen retention is not usually directly proportional to the amount of renal damage. A considerable experience has shown that ureteric and urethral obstruction produces much greater nitrogen retention than the most marked degenerative change. In view of the lack of a quantitative relationship between nitrogen retention and renal damage, various other constituents have been held to indicate the conditions more clearly. Thus Myers (4) and his co-workers hold that the estimation of creatinin content is more reliable. They state that a creatinin content constantly over 1·5 mg. per 100 c.c. is a sign of permanent renal damage. That this is true cannot be doubted, but a number of cases have been examined with marked renal inefficiency with a normal creatinin content.

In nitrogen retention, all the bodies mentioned above increase in the blood, and if one estimates urea, which is present in the greatest concentration, and non-protein nitrogen, which, as its name implies, embraces all the soluble nitrogenous bodies exclusive of protein, fallacies are excluded in so far as is possible.

The question of normal variations in the blood urea concentration is held by some as an objection to this criterion, yet so experienced an observer as Otto Folin states that these do not come within the sphere of practical politics.

It is safe to conclude, therefore, until more evidence is brought forward, that the blood urea and non-protein nitrogen content form one of the best guides, both to prognosis and to diagnosis. If these bodies be present in con-

centration over 40 mg. per 100 c.c., we may assume there is renal inefficiency provided that the reservations made above are observed. As will be pointed out later (p. 33), the calcium content of the blood usually falls from the normal of 10 mg. per 100 c.c. to 6 mg. or lower, and the phosphorus rises from a normal of 2·5 mg. per 100 c.c. to 5 mg. or over during the terminal stages of nephritis, particularly if there be nitrogen retention. These changes indicate a grave prognosis. Marked nitrogen retention occurs mainly in chronic nephritis with no œdema (chronic interstitial nephritis) and in cases where there is obstruction to the urinary flow. Little or no retention is found in chronic nephritis with œdema and lipoid nephrosis until the terminal stages, hence these cases are not usually detected by an examination of the blood nitrogen distribution. Many tests are described for their identification, but perhaps the most important are :—

(*a*) The estimation of blood cholesterol ; and

(*b*) The estimation of the albumin-globulin ratio in the blood.

(*a*) Albert Epstein (5) has employed the estimation of blood cholesterol as a means of diagnosis, and also as a method for checking the treatment of lipoid nephrosis. Normally the blood cholesterol lies somewhere about 0·18%, whereas in this condition it is markedly increased, up to as much as 0·3% or over. With suitable treatment, such as a high protein diet and the administration of thyroid extract, the cholesterol content falls as the case improves clinically. Admittedly there are many other conditions which can influence the blood cholesterol content, such as gall-stones, but these can usually be excluded by clinical examination. We have used this method extensively and found it very satisfactory.

Whilst it used to be maintained that the blood chlorides were increased in nephritis with œdema, the importance of this estimation is not considered very great at present. A diagnostic change in the blood chlorides is rarely, if ever,

seen. That actual retention of chlorides in the body as a whole occurs cannot, however, be doubted, since the urinary chlorides may practically disappear in a case with severe œdema.

(*b*) The method for the estimation of the serum albumin and globulin is given on p. 395. In normal subjects the albumin is about 5·6%, whilst the globulin is about 1·9%, the total protein being about 7·5%. In chronic nephritis with œdema the albumin may be reduced to 2·5%, but the globulin will only fall to about 1·7%, and the total protein will be reduced to just over 4%. This change is characteristic of lipoid nephrosis and of nephritis associated with œdema and massive proteinuria. As already pointed out, the urine in such cases contains a greater proportion of albumin to globulin, from which it may be concluded that the relatively low blood albumin is due to its excretion into the urine.

If this state of affairs be found, namely a reduction of the total protein and a relative increase in the globulin in the blood, it is an indication that the case will probably do well and that the œdema will disappear on a high protein diet. Epstein (6) has advanced the view that the œdema is due to the excretion of proteins into the urine, thus robbing the blood of its normal indiffusible colloids with a consequent reduction in osmotic pressure. To compensate this, fluid passes from the blood into the tissues, thus producing œdema. A high protein diet restores the blood proteins and thus increases the osmotic pressure of the blood and draws fluid from the tissues.

In conclusion, it must be thoroughly understood that the figures obtained by blood analysis can only be taken as a guide. The estimation of phosphates in blood has been recommended by de Wesselow (7), as giving more reliable results than those obtained by blood nitrogen estimations. This work will be referred to in Chapter XV. It was found that the phosphate content of plasma was increased in cases of chronic nephritis with nitrogen retention.

Renal Efficiency as Judged by Quantitative Analysis of the Urine

The Volume and Specific Gravity.—The character of the urine in various renal diseases is so well known that it is only necessary to summarise very briefly the conditions.

	Vol. in c.c.	*S.G.*
Normal urine . .	1,500	1,015 to 1,025
Acute nephritis .	200 to 500	1,025 to 1,035
Chronic nephritis with no œdema	2,000 or over	1,005 to 1,012
Lipoid nephrosis .	600 to 900	1,020 to 1,025

Rowntree and Fitz (8) state that the actual volume of urine secreted over a given period of time bears little or no relationship to the renal function, although they have constantly observed that the specific gravity is markedly decreased in advanced cases of kidney disease.

Investigation of the Various Constituents.—There is a marked alteration in the composition of the urine in severe nephritis, and it would be reasonable to hope that valuable information concerning the renal efficiency would be yielded by urinary analysis. It is impossible to over-emphasise certain fallacies in this method. Firstly, the diet must be known, and preferably should be of some standard type. Without a knowledge of the protein intake, no inference can be drawn from the urea excretion, since the two vary directly with each other. A possible exception to this rule is when one desires to differentiate between the nitrogen retention due to renal disease, and that due to some abdominal condition such as vomiting or obstruction. As already pointed out, in the former case the urinary urea will be well below 2%, and in the latter, probably 2% or over. Secondly, no reliance must be placed on an analysis of less than an accurate twenty-four hours' specimen. The analysis of isolated specimens of urine is a waste of valuable time. For

instance, in normal people, a glass of beer half an hour or so before the specimen was collected would be quite sufficient to lower the urea content to 1% or less.

Tests of Renal Efficiency which depend on the Relative Concentration of Substances in the Blood and Urine

The difference between the specific gravity of blood and urine attracted the attention of physiologists many years ago, and the concentrating powers of the kidney were soon recognised. These physiological investigations led on to pathological ones, and it was soon discovered that the concentrating power of the kidney was altered in certain forms of nephritis. Thus it was noted that the specific gravity of the urine was markedly decreased in cases of chronic interstitial nephritis.

Ambard (9) was certainly the first observer to work out a scientific application of these phenomena. He tried to obtain a ratio between the blood and urinary urea, taking into account the body weight and the length of time during which the observations were made. The result was arrived at by a mathematical equation, and was known as Ambard's co-efficient.[1]

Although valuable information can be obtained by this method it is open to many fallacies, and has been criticised by many able workers. Thus Folin considers that the observation period, seventy-two minutes, is far too short,

[1] Ambard's co-efficient is calculated from the formula :—

$$\frac{m}{\sqrt{D \times \frac{70}{wt.}} \times \sqrt{\frac{c}{25}}} = \text{constant},$$

where m = grams of urea per litre of blood.
D = grams of urea excreted per twenty-four hours.
wt = weight of individual in kilograms.
c = grams of urea per litre of urine.

Normally the value is about 0·08, with impairment of function there is a rise.

and Addis and Watanabe (10) state that, as the results of their experiments on normal subjects, urea excretion depends on factors other than the concentration of urea in blood and urine.

GROUP III

TESTS OF RENAL FUNCTION DEPENDING ON THE ELIMINATION OF SOME SUBSTANCE ADMINISTERED TO THE BODY

Natural Substances

1. *Water.*—Under this group is included the provocative polyuria test.

The Straus-Graunwald method (11). Here a pint of water is given to a starving patient and the urine is collected at hourly intervals. In the case of a normal person the sum of the first three hours' specimens should equal the quantity of fluid taken, whereas if the patient be suffering from nephritis the quantity is considerably less.

It is, perhaps, a pity that tests on similar lines to that described above are not performed more often. It would be expected that they would yield valuable information, since the work of Rowntree (16), and his co-workers, on water intoxication emphasises the importance of this factor.

2. *Urea.*—The administration of urea, followed by observations on the urinary excretion, has been used extensively as a test of renal function. McKaskay (12), in America, has employed this type of test, giving 30 g. of urea, and analysing the urine for urea at two-hourly intervals for twenty-four hours after. MacLean (13), in this country, employs this principle in his well-known urea concentration test, which is designed to estimate renal function by the concentrating power of the kidney. After emptying the bladder the patient is given 15 g. of urea dissolved in 5 oz. of water flavoured with tinct. aurantii m.xv, and the urine is collected one hour and two hours afterwards. For a child of twelve years or upwards the full dose is given. Between the ages of three and

five, half a dose is required, and proportional amounts are given to children above or below this age period. The specimens are then analysed for urea by the hypobromite method. According to MacLean, in a normal person the first specimen contains at least 1·5%, whilst the second should have a minimum concentration of 2%. Figures below these show a poor concentrating power of the kidney and indicate renal inefficiency. The test depends on flooding the blood with urea, thus raising its concentration above the normal value, and observing how rapidly the kidney removes the excess. Like all tests in which absorption of substances from the alimentary canal plays a part, it suffers from the disadvantage arising from uncertainty in this direction. Thus the urinary excretion of urea depends in this case, not only upon the concentrating powers of the kidney, but also upon the rate of absorption which is controlled by the emptying time of the stomach, since urea is absorbed only from the intestines. MacLean, however, has used the test in over 100,000 cases, and very valuable information has been obtained with its aid. The urea concentration test is used extensively in this country.

3. *Renal Test Meals.*—This method was introduced with the hope of estimating renal function by analysis of short period samples of urine following a standard diet. Mosenthal (14), who has had, perhaps, the greatest experience with this type of test, employs a standard meal and collects two-hourly specimens of urine throughout the day. If the standard diet cannot be employed, the patient may have three good meals in the day, one at 8 a.m., another at 12 o'clock, and the last at 5 p.m. The bladder is emptied at 8 a.m. after a night's fast, and the urine is collected at two-hourly intervals until 8 p.m., and the night urine is collected in one quantity from 8 p.m. to 8 a.m. The specimens are then examined for volume, specific gravity, urea and salt concentration.

With regard to the interpretation of these figures, Mosen

thal lays stress on the following points as indicating normal secretion :—

1. Variations in the specific gravity of the urine—nine points, or even more.

2. Rough balance between intake and output of salts and nitrogen.

3. High specific gravity of the night urine, with a high nitrogen content (1% or over) : volume moderately small (400 c.c.). The volume of the night urine bears little or no relation to the amount of fluid ingested.

The response of a nephritic individual is quite different from the above. One of the most noticeable points is the fixation of the specific gravity. The nephritic kidney tends to secrete a urine of persistently low specific gravity, despite the amount of food and water given. According to Mosenthal renal impairment is indicated by the following signs :—

1. Fixed or low specific gravity.
2. Lowered output of salts and nitrogen.
3. Tendency to total polyuria.
4. Loss of concentration in the night urine, associated with low specific gravity and nitrogen contents.

The response of all kinds of renal patients to this test meal has been worked out, and the results can be seen from Mosenthal's work. Space, unfortunately, forbids a fuller account of this method, which appears to have given such success in America, and more work on these lines might profitably be conducted in this country.

Foreign Substances, such as Dyes

There are two important tests in this group :—

1. The indigo carmine test ; and
2. The phenol sulphone phthalein test.

1. *The Indigo Carmine Test.*—The use of indigo carmine was first suggested by Heidenhein, but the actual working out of the reaction was performed by Volcker and Joseph (15) in 1903.

Ureteric catheters are passed and 0·1 g. of the dye is injected intramuscularly. The urine should be tested after six to eight minutes. The intensity of the colour will indicate roughly how much of the dye is excreted, and any delay in its appearance will indicate renal inefficiency. The test is especially useful in determining which kidney is affected, and finds particular use in surgery. The excretion is usually complete in twelve to twenty-four hours.

2. *The Phenol Sulphone Phthalein Test.*—This reaction was first described by Rowntree and Geraghty (16) in 1910, and since that date has found very extensive employment.

The patient is given 300 c.c. of water to drink, and twenty minutes later the bladder is emptied and 6 mg. of the dye in 1 c.c. of sterile saline are injected intramuscularly. It is as well to employ a catheter, and to let the urine drip out after the injection into a test tube containing a dilute solution of liquor potassæ. As soon as the dye appears in the urine, the solution in the test tube turns red : this should happen within ten minutes of the injection. The catheter is then withdrawn, and the whole of the first and second hour's urine is collected separately. The samples are measured, and enough 25% NaOH solution is added to each to produce a deep red colour, and the volume of each is made up to a litre. A standard solution is prepared by dissolving 0·003 g. of the dye in water and adding 25% NaOH solution to produce a permanent colour. The volume is then made up to 1 litre, and the known and unknown solutions are compared in a colorimeter. The amounts of dye in the first and second hour's specimens are calculated according to the methods described in the section on blood analysis. By the first hour 50%, and by the second hour 70% of the drug should be excreted. Anything below this points to renal inefficiency. By ureteric catheterisation the test can be applied for localising the lesion to one or other kidney.

The test is very satisfactory provided that there is no blood in the urine, and is of value in surgical cases.

In conclusion, it is perhaps advisable to add a few remarks concerning the relative value of the various tests described in this section. It can be readily understood that no one test will give equally satisfactory information in all cases of renal inefficiency, and, consequently, one must choose those which are especially suited to the particular type of case under examination. Again, the value of the information obtained unfortunately differs according to the type of case investigated.

In routine hospital work, renal problems fall under the following headings :—

1. *The Surgical Case.*—Can a prostate be removed with safety ; or, if the right kidney be removed, will the left be sufficient for the patient's needs ?

2. *The Obstetric Case.*—Is it safe to let a woman with albuminuria go to term ?

3. *The Medical Case.*—What is the prognosis of a young adult with albuminuria ?

Very satisfactory results are obtained in Group I., quite good in Group II., but it must be admitted that the greatest number of failures are encountered in Group III.

The Surgical Case.—Here one is usually required to express an opinion on prostate cases, or on patients showing localised kidney lesions or urinary obstruction. For the former type, blood analysis has yielded by far the most accurate results. The urea alone cannot be relied upon, since it is possible to have marked nitrogen retention with a normal blood urea. The best guide is found to be the urea and non-protein nitrogen contents. Experience has taught us that any figures much over 50 mg. per 100 c.c. of either urea or non-protein nitrogen are contra-indications for prostatectomy in one stage. We have seen fatal results where the operation has been performed with either the blood urea or non-protein nitrogen contents over 60 mg. per 100 c.c. If figures of 60 mg. per 100 c.c. or over be found, it is well to perform the operation in two stages—suprapubic

cystotomy — followed by prostatectomy after a week or so. If an analysis be done a week after the first operation, the figures will be found to have decreased remarkably. In one particular case investigated in the Courtauld Institute of Biochemistry, the blood urea and non-protein nitrogen content before suprapubic cystotomy were 125 and 140 mg. per 100 c.c. respectively, whilst ten days after the operation they had fallen to 48 and 54 mg. per 100 c.c. In many cases the figures will have fallen to within the safe limit seven to ten days after suprapubic cystotomy. The results obtained from tying in a catheter are not nearly so good. The nitrogen figures improve at a very much slower rate, which is in agreement with the clinical surgical view on the relative advantages of these two methods. It must be understood that the above account is based upon the authors' experiences, and that satisfactory results have been reported by the employment of other tests described in this section, but blood analysis has been found by us to give the most reliable information. In the other types of surgical cases encountered, dye excretion tests are by far the most satisfactory, and are usually performed by the surgeon himself. Thus the indigo-carmine test is used almost exclusively for determining disturbances of one or other kidney or ureter.

The Obstetric Case.—This variety of renal problem is constantly arising.

A woman, six months pregnant, has albuminuria with or without clinical symptoms, such as headache, vomiting or œdema. Should she be allowed to proceed to term, or should the pregnancy be terminated by induction of premature labour? A vast amount of work has been done on the renal side of the toxæmias of pregnancy, and it is difficult to epitomise the views of workers in this field. The following scheme has been found to embrace most of the problems arising in this group of cases, and is taken from a paper by Comyns Berkeley, A. L. Walker and one of us (E. C. D.) (17).

The albuminuria of pregnancy can be classified according to the following scheme :—

1. Cases of albuminuria with no clinical symptoms.

2. Cases of albuminuria with clinical symptoms, such as headache, vomiting, œdema, etc.

3. Cases of albuminuria with actual or threatened eclampsia.

In Group I., there is little or no nitrogen retention, and no chemical evidence of liver inefficiency (see Chapter V., on hepatic efficiency). These patients go to term normally, and are probably cases of functional albuminuria, or, according to some authorities, show minor symptoms of raised blood pressure.

Group II. must be divided into two sub-divisions :—

(*a*) *The Nephritic Toxæmia*, as de Wesselow names cases of pregnancy in chronic nephritis. Here there is marked nitrogen retention, with a blood urea and non-protein nitrogen of over 40 mg. per 100 c.c., a high blood pressure and history of chronic nephritis.

In this type of case pregnancy should be terminated as soon as the blood urea and non-protein nitrogen figures rise much above 40 mg. per 100 c.c. Although it is possible for such cases to go to term, experience has shown that their renal condition is much worse after delivery than before.

(*b*) *The Pre-eclamptic Toxæmia or Pregnancy Kidney.*—In these cases there may be little or no nitrogen retention. If treated suitably by dieting, etc., these patients go to term without passing into Group III. The possibility of eclampsia, however, should never be overlooked. Blood analysis will not, unfortunately, provide an infallible means of determining whether eclampsia is imminent. It must be admitted that the urea and non-protein nitrogen figures usually rise before the onset of eclampsia, yet it is quite common to find figures of about 40 mg. per 100 c.c. for the blood urea and non-protein nitrogen of an eclamptic patient. As will be explained in Chapter V, dealing with hepatic tests, the onset

of eclampsia is heralded by the appearance of bile pigments and their precursors in the blood and urine.

Group III., or cases of eclampsia, are recognised clinically and treated immediately. There is always evidence of renal inefficiency in eclampsia. Thus there is albuminuria, diminished urea output, and frequently an increase in the blood urea. The non-protein nitrogen content is always above normal. Perhaps the most important point to realise is that it is impossible to judge the imminence of eclampsia from the amount of nitrogen retention.

To summarise the scheme, premature labour should be induced if—

(*a*) The blood urea and non-protein nitrogen contents are above 40 mg. per 100 c.c. (nephritic toxæmia).

(*b*) The hepatic tests become positive (pre-eclamptic toxæmia changing to eclampsia).

The Medical Case.—The real difficulties of renal function testing lie in this type of case. In surgery and obstetrics it is sufficient to say that such and such a patient's kidneys are so severely damaged that an operation should not be performed, or that pregnancy should be terminated. In other words, whilst no diagnosis is required, it is sufficient to state whether or not the excretory powers are adequate. In medicine, however, matters are very different. It is of little value to inform a physician that his patient has some sort of renal lesion with impaired efficiency, facts which can usually be elicited by a purely clinical examination. If the problem of a young adult with albuminuria be considered from the point of view of prognosis, it is easy to see the difficulties in the way. All tests will probably show slight impairment of function—yet they do not give enough information to be of any particular value to the clinician. Again, if the patient be œdematous, the physician wants to know whether to give a high or low protein diet. Blood analysis will help him in this respect. With regard to blood analysis in general in relation to medical cases,

the results are somewhat disappointing. Thus the amount of nitrogen retention seems to bear little or no relation to the extent of renal damage. It has been found that obstruction to the urinary flow produces much greater nitrogen retention than the most severe kidney lesion. Some observers state that three-fourths of the kidney must be destroyed before nitrogen retention appears.

It has usually been claimed that blood analysis will indicate whether a particular patient is likely to go into uræmia. Although this is true in the great majority of instances, we have seen four cases in one year with typical uræmia and blood urea contents below 40 mg. per 100 c.c. The non-protein nitrogen content, however, was high in all. The estimation of blood calcium and phosphorus is certainly of value in this connection. Thus, a reduced calcium and increased phosphorus content are almost invariably associated with uræmia, and indicate a grave prognosis.

Andrewes' Reaction.—Of recent years there has been considerable interest in a chemical test on the blood for the diagnosis of uræmia. This was first described by Andrewes (18), who noted that if the van den Bergh reaction were performed on serum obtained from a uræmic patient, a cherry-red colour could be obtained by adding concentrated alkali after performing the indirect reaction. A simple modification of the test has been described by Harrison and Hewitt (19), whose method is as follows :—

The ordinary reagents as used in the van den Bergh test, described on p. 151, are employed. To one volume of serum, two volumes of absolute alcohol are added and the precipitated proteins are removed by filtration. The authors suggest that the extract may be improved by heating the mixture in a boiling water bath prior to filtration. Four volumes of the filtrate are taken and one volume of the diazo reagent is added. The resulting mixture is boiled for a half to one minute and 40% caustic soda solution is added, drop by drop, shaking after each addition. A

positive reaction is indicated by the development of a definite pink or cherry-red colour. This is fleeting and may only last a few seconds. In a very severe case of well-established uræmia the colour may last as long as half an hour.

There has been a great deal of discussion as to the substance responsible for this reaction, but it would appear that this has definitely been settled by Harrison and Bromfield (20), who state that the reaction is given by an indoxyl compound, presumably potassium indoxyl sulphate or, alternatively, indoxyl glycuronate. It would appear, therefore, that it is a test for indican in the blood. The reaction is given by alcoholic extracts of normal urine.

A series of observations have been made in order to assess the clinical value of this test (21). The conclusions of the last-named observers were that the reaction is only found " in uræmia or severe renal inefficiency. A negative reaction, however, does not exclude uræmia." They state that nitrogen retention always accompanies the positive reaction, but that retention of indican does not necessarily run parallel with that of the nitrogenous bodies.

It would appear, therefore, that this test may afford valuable information in differential diagnosis, but it is at present too early to rely upon it entirely. It must also be borne in mind that uræmia is a very complex clinical condition exhibiting many varied symptoms, and that on general principles one would imagine it to be almost impossible to obtain a chemical test for its diagnosis. Further observations will indicate whether this reaction may be used in a prognostic sense.

Blood analysis is particularly useful in checking the effects of dieting on nitrogen retainers. Under suitable treatment, marked reductions can be made in the blood urea and non-protein nitrogen contents.

With regard to lipoid nephrosis, the estimation of the cholesterol content has been found to be of particular value.

The normal figure is about 0·18%, whilst in this type of nephritis it is very frequently above 0·3%, as already pointed out. With efficient treatment, this figure can often be reduced.

In conclusion, then, it may be said that renal function tests, considered from the medical aspect, fall short in the fact that they do not provide a diagnosis, and give a poor idea as to prognosis. This applies equally to all tests. Nitrogen retention can best be diagnosed by blood analysis. If there be marked nitrogen retention, say, blood urea and non-protein nitrogen contents of about 100 mg. per 100 c.c., it is always well to exclude the possibility of urinary obstruction before regarding the case as one of serious prognosis. Figures of the magnitude quoted above, found on repeated examination, are to be regarded seriously if there be no stricture and no condition such as intestinal obstruction, but if due to either of these causes they will fall to normal in a week or so after the removal of the cause, always provided that there is no serious permanent renal damage.

A low urinary diastase content is held by many to be a sign of renal damage. We have not found this test to give constant results.

Whatever test, or series of tests, be adopted, the importance of clinical examination must never be lost sight of, and the blood pressure, condition of the arteries, etc., are still of the greatest value. It is only by means of the closest co-operation between the physician and biochemist that one can hope to gain knowledge of real value in the study of deranged renal function.

Types of Nephritis and their Treatment

The older clinical and pathological classifications of the types of nephritis have not stood the test of practical experience, and thus lead to confusion. At present, no satisfactory clinical classification based purely on post-mortem

findings appears possible. We are therefore purposely omitting a description of the pathological changes in the kidneys, and do not intend to discuss any of the elaborate classifications based on the type of lesion thought to be present during life, which are so often stultified at the autopsy. Further, from time to time, the post-mortem examination reveals that there is suppuration in the kidney, in cases thought during life to be suffering from nephritis.

Our aim is to describe the case as it presents itself to the practitioner and indicate the facts which enable him to allot it to a simple clinical type. The chief clinical and biochemical findings are described in order to enable this to be done. Treatment is next considered and indications are given for special diets, such as a high, medium or low protein or a salt-poor one, representative diet sheets being included. The use of special drugs in nephritis is also considered and a clinical case sheet is appended which is useful for systematic records, and prevents important data from being overlooked in individual cases.

The following clinical types of nephritis are easily recognisable :—

(1) Acute nephritis. (2) Subacute nephritis. (3) Chronic nephritis with œdema. (4) Chronic nephritis with slight or no œdema. (5) Mixed types of chronic nephritis.

Acute Nephritis

Acute Diffuse Glomerulo-Nephritis.—*Clinical Findings.*—The disease has a definite onset, and is thus easy to diagnose. The cases fall into groups according to the prominence of the initial symptoms.

(*a*) *Hæmorrhagic.* Hæmaturia is the first abnormality noticed. This type of nephritis was common in the Great War. (*b*) *Œdematous.* Swelling of the extremities, face and body are early symptoms. (*c*) *Toxæmic.*—The patient first notices some of the following symptoms: headache,

backache, nausea, vomiting, diarrhœa, malaise or dyspnœa. A convulsive attack may be an early symptom. Frequently a combination of these groups of symptoms is noted; thus the patient may complain of his legs swelling and of passing dark urine. The heart is not enlarged. The blood pressure rises early in the illness and falls later as diuresis sets in. Retinal changes, as narrowing of the arteries, dilatation of the veins, hæmorrhages and œdema of the disc, may be found. Convulsive uræmic symptoms are especially liable to occur.

Course. The nephritis may clear completely in a few weeks, or pass into a subacute stage. The latter may resolve or become chronic, either with or without œdema. Death may rapidly occur from anuria with uræmic manifestations.

Biochemical Findings. The Urine.—Volume diminished, 10–20 oz. (300–600 c.c.). Sp. Gr. 1,025–1,035. Protein 20–80 g. per litre. Chlorides reduced. *Cells*, red and white blood cells and renal epithelial cells. *Casts*, hyaline, blood and epithelial.

The Blood.—Urea and the non-protein nitrogen are often raised to about 60 or 80 mg. per 100 c.c. Chlorides, normal. Cholesterol, normal. Protein, the amount of albumin and globulin is normal. Total protein is about 7·5% of which the albumin is about 5·6% and globulin about 1·9%.

Urea concentration test, low values.

Water elimination test, low values.

Treatment.—The ordinary "milk" diet should not be given, as it contains too much fluid and too much protein. The following diet may be substituted :—

Days 1 to 3. Total fluid not more than 1 to 1½ pints, composed of water, barley water, imperial drink, glucose orangeade or lemonade (8 oz. of glucose to 1 pint, with the juice of one orange or lemon). No solids must be taken. *Days 4 to 7.* After diuresis has occurred, or when the hæmaturia has diminished, the diet may be increased. Milk ½ to 1 pint, glucose orangeade 1½ pints, a little toffee, thin bread and

butter, Benger's food, cream ½ oz. Sugar and weak tea. *After 8 days.* The diet is again amplified. Milk 2 pints, bread and milk, sugar, toast, bread and butter, honey, cream, potatoes. Later may be added milk pudding, suet pudding, porridge, shreaded wheat, bananas, steamed fish, chicken, boiled lamb, and one egg a week. No salt should be used in cooking nor taken with the food while any œdema persists.

In addition to the diet, the recognised treatment includes rest in bed between blankets or wool sheets, the administration of saline aperients, hot applications or dry cupping to the loins, and hot air baths, to induce sweating. Diuretic drugs should not be given, at any rate until diuresis has begun. Urine elimination may then be increased by the use of such drugs as are given on p. 42.

For convulsive uræmia sedatives as chloral hydrate gr. 30 in 2 oz. water may be given per rectum, or magnesium sulphate may be prescribed in doses of ½ to 1 dr. by mouth, repeated every two hours, or 14½ dr. in 8 oz. water (= 25% solution) per rectum, repeated in an hour's time if necessary. Other measures include venesection, lumbar puncture, or intravenous injection of 40 c.c. of 30% sodium chloride.

Focal Glomerulo-Nephritis.—This is a special type of acute nephritis. When there is hæmaturia, and no œdema or signs of renal insufficiency, as judged by the renal function tests, occur subsequently, the condition is diagnosed as focal glomerulo-nephritis. Only a few glomeruli are thought to be involved. Children are often affected by this type of nephritis, which is usually a complication of a streptococcal sore throat. It may also occur during the first week of scarlet fever, or as a complication of otitis media, septicæmia, erysipelas or acute infective endocarditis. When a cryptic septic focus persists, as in a tooth or tonsil or cranial sinus, recurrent attacks of hæmaturia may ensue, especially after exposure to cold or when the patient is in a lowered state of health.

Treatment.—No special treatment is usually required for

the kidney. The patient is kept in bed, and if the urine be scanty the fluid taken by the mouth should not be more than 1½ to 2 pints. As soon as diuresis sets in plenty of fluid should be drunk. When the acute symptoms have subsided, any septic focus should be dealt with.

Embolic Glomerulo-Nephritis.—This is a complication of subacute infective endocarditis, and is characterised by hæmaturia and albuminuria. Hyaline, granular and blood casts may be found in the urine. The blood pressure does not rise, there is no œdema and no renal insufficiency.

Treatment.—No special measures are necessary for the kidney ; the treatment is that of the associated heart lesion.

Subacute Nephritis

Clinical Findings.—Subacute nephritis may be a sequel to an attack of acute nephritis, the patient remaining œdematous, and red cells persisting in the urine for more than two months after the onset of the acute attack. It may also be due to a recrudescence of activity in a case of chronic nephritis.

Course.—When directly ensuing on acute nephritis it may completely resolve ; frequently, however, the nephritis becomes chronic, either with or without œdema, proving fatal in a few months or years. The blood pressure tends to remain raised and the heart gradually enlarges. Acute convulsive uræmia or later chronic uræmic symptoms are noted. Retinal hæmorrhages, exudates and choked disc are prone to form.

Biochemical Findings.—The Urine.—Volume diminished. Sp. Gr. rather low. Protein, a trace to 20 g. per litre. Chlorides, reduced. *Cells*, a few red cells, leucocytes, epithelial cells and fat granules. *Casts*, a few blood, epithelial, hyaline or granular ones.

The Blood.—Urea and the non-protein nitrogen tend to rise, and may reach a terminal figure of about 400 mg. per

100 c.c. in a progressive case. Cholesterol, normal. Protein, normal.

Urea concentration test, low values.

Water elimination test, low values.

Treatment.—The patient should be kept in bed with blankets or wool sheets.

Diet.—A "salt-free" diet may be used. This implies a salt-poor diet as every article of food contains a certain amount of sodium chloride. No salt must be used in cooking, and none added to the food when eaten. A strict Karrell diet, which is salt-poor, may be given. This is as follows: *Days* 1 *to* 7. Milk 7 oz. at 8 a.m., 12 noon, 4 p.m. and 8 p.m. Total salt is 1·3 g. *Day* 8. Add at 10 a.m. a softly cooked egg and one slice of toast. Total salt is 1·78 g. *Day* 9. Add 2 oz. of vegetables, as asparagus, celery, cauliflower or carrot, and two teaspoonsful of starch to the milk taken at noon, to form milk soup. Add one slice of toast at 4.30 p.m. Total salt is 1·89 g. *Days* 10 *to* 12. Add one egg, 1 oz. rice, weighed raw, and 2 oz. of the vegetables. Total salt is 2·41 g. Substances relatively poor in salt may be taken later, as bread made without salt (0·01% NaCl), salt-free butter and oatmeal (0·02% NaCl), stewed fruit (0·03% NaCl), fresh water fish (0·04% NaCl), oysters (0·05% NaCl), and potato (0·06% NaCl). Milk contains 0·16% NaCl, as do also chicken, steak, mutton, veal, lamb, and yolk of egg. Sea fish, white of egg and ordinary bread contain 0·5% NaCl.

If the œdema does not respond to a salt-poor diet, other measures may be tried, such as the alkali treatment, diuretic treatment and fluid drainage.

The Alkali Treatment.—An ordinary mixed diet, containing a moderate amount of protein, such as 60–80 g., is taken. This conforms with the basal requirement of $\frac{1}{2}$ g. of protein per 1 lb. body weight. The fluid is not restricted, but the amount taken should not be much greater than the volume of urine passed.

Moderate Protein Diet

	Grams.			Calories.
	Protein.	Fat.	Carbo-hydrate.	
Breakfast—				
Egg, 1	6	6	0	78
Bread, 4 oz.	8	1·6	57	276
Butter, ½ oz.	0	12·5	0	112
Milk, 4 oz.	4	4	6	76
Marmalade, ½ oz.	0	0	9	36
Tea, 5 oz.	0	0	0	0
Lunch—				
Fish, 3 oz.	15	0	0	60
Potato, 4 oz.	0	0	20	80
Green vegetables, 2 oz.	0	0	2	8
Stewed fruit, 2 oz. (weighed raw)	0	0	8	32
Butter, 1 oz.	0	25	0	225
Bread, 4 oz.	8	1·6	57	276
Tea—				
Bread, 2 oz.	4	0·8	28·5	138
Butter, ½ oz.	0	12·5	0	112
Jam, ½ oz.	0	0	9	36
Milk, 2 oz.	2	2	3	38
Tea, 5 oz.	0	0	0	0
Dinner—				
Chicken or lamb, 4 oz.	24	12	0	204
Potato, 4 oz.	0	0	20	80
Green vegetables, 2 oz.	0	0	2	8
Fruit, 4 oz.	0	0	16	64
Bread, 2 oz.	4	0·8	28	138
Sugar for sweetening during day, 2 oz.	0	0	56	112
	75	78·8	321·5	2,189

The alkali treatment recommended by Osman (22) is as follows: The patient is kept in bed and weighed every third day. The amount of urine passed every twenty-four hours is measured. The reaction of the morning urine, passed

before breakfast, is determined colorimetrically as described on p. 79 while it is still fresh. Diuresis does not usually occur until the urine is alkaline to phenol phthalein (*p*H 8·3 or over). The plasma bicarbonate is determined before treatment is started, and subsequently during treatment. The following mixture is given: Pot. cit., pot. bicarb., sod. cit and sod. bicarb. gr. 15 of each, sp. chlorof. m. 7, and aq. menth. pip. to the ounce, three times a day. The total amount of alkali is therefore 180 gr. in the twenty-four hours. The alkali is increased by 60 or 100 gr. daily until the *p*H of the morning urine specimen reaches about 7·6. This may occur with a daily dosage of 760 gr. The œdema often increases at this stage, as shown by the weight of the patient; the plasma bicarbonate should be estimated again and the dose of alkali increased further until diuresis ensues, which may occur when the daily dosage reaches 960 gr. This amount is given daily until the œdema disappears and the volume of the urine falls to normal. There is a risk of tetany at this stage, owing to alkalosis, and in children diarrhœa is liable to occur. Tetanic symptoms can be controlled by giving calcium chloride gr. 1 in m. 40 distilled water intramuscularly every two or four hours. When the œdema has disappeared, which may take ten days to three weeks, the alkali is gradually reduced by 100 gr. every two or three days, providing there is no return of the œdema. The albuminuria usually diminishes with the œdema. If there be return of the œdema the dose must be increased again. The patient usually has to take about 240 gr. daily for two or three weeks subsequently. This method should only be used when the patient is in hospital and under laboratory control.

Diuretic Treatment.—Various other diuretic drugs can be used, combined with a moderate protein and low fluid diet. These include theophylline (theocin sodium acetate) gr. 2 to 4, t.d.s., in a cup of tea for three days, diuretin (theobromine sodium salicylate) gr. 5, t.d.s., for three days, caffeine gr. 2, t.d.s., digitalis and mercury as Guy's diuretic pill, 1, t.d.s., and urea 15 g. (255 gr.) in 5 oz. of water, flavoured with syr. aurantii t.d.s. When such large doses of urea

are given the blood nitrogen should be estimated daily to see that it does not rise unduly.

Novasurol[1] is a water-soluble drug containing 33·9% mercury. It produces diuresis in cases of œdema or ascites due to affections of the heart, liver or kidneys. Keith, Barrier and Whelan (23) have reported a series of cases of nephritis of varying types treated with novasurol, the usual dose being 1 c.c. of a 10% solution. This contains approximately $\frac{1}{2}$ gr. of mercury. In our experience novasurol may be used in cases of nephritis, provided adequate caution is exercised with the initial and subsequent doses. Its efficacy as a diuretic is increased by the simultaneous administration of ammonium chloride.

0·5 c.c. of a 10% solution of novasurol is injected intramuscularly to see that there is no intolerance, as shown by hæmaturia, increased albuminuria or diarrhœa. Ammonium chloride, gr. 30, is then given by mouth every six hours, either in gr. 10 capsules, or as a mixture containing ammon. chlorid. gr. 30, ext. glycyrrhiz. liq. m. 20, sp. chlorof. m. 7, and aq. ad. 1 oz. One cubic centimetre of the novasurol is injected intramuscularly next day, and is repeated every three to seven days, the ammonium chloride being given throughout the treatment. Diuresis usually occurs between three and twelve hours after each novasurol injection, and ceases within twenty-four hours. If no diuresis occur after the second injection it should be discontinued.

Purgatives.—Drugs causing a watery evacuation, such as pulv. jalapæ co, ℥i, or mag. sulp. ℥ii, or sod. sulph. ℥ii, are of value in assisting to reduce the œdema.

Fluid Drainage.—If these measures fail to relieve the

[1] Novasurol is a double compound of sodium mercuri-o-chlor-phenyl-oxy-acetate and diethyl-malonyl-urea. Another substance, salyrgan, is less liable than novasurol to produce toxic effects. It is a complex compound of mercury and sodium salicyl-allyl-amino-o-acetate, and contains 33·5% mercury. We have found that salyrgan produced no toxic effects when given intramuscularly or intravenously in one of our cases of chronic nephritis with œdema, in which a test dose of novasurol resulted in severe diarrhœa and melæna. The intravenous salyrgan produced good diuresis, whereas a previous intramuscular injection had failed. It is administered in similar doses as described above for novasurol, and ammonium chloride should be given simultaneously by mouth.

œdema, the excess of fluid may be removed from the body to a certain extent by incisions in the legs or by tapping the peritoneal or pleural cavity.

Chronic Nephritis with Œdema

Chronic nephritis with œdema may follow directly or indirectly upon an attack of acute nephritis, or it may have an insidious onset. When called on to investigate or treat such a case, it is best to determine first whether the patient is suffering from lipoid or amyloid nephrosis. If it be found that the kidney lesion is not of these types, it is considered to be one of chronic diffuse glomerulo-nephritis.

Lipoid Nephrosis. This name was first used by Müller (24) in 1905, applied to renal degeneration, the changes being especially in the tubular epithelium. Widespread recognition of this group of cases is due particularly to the work of Epstein (25), and his name is especially associated with his mode of treatment described on p. 45.

Clinically there appear to be two types: *Primary lipoid nephrosis.* The disease occurs in young adults, and has an insidious onset with malaise, headache, pallor, œdema, and perhaps nausea and diarrhœa. The heart is not enlarged, the blood pressure is not raised, and the basal metabolic rate is usually low. Retinal changes do not occur. Complete recovery is said to be the rule, but we have not met with any case of this type. A glomerulo-nephritis may supervene, as shown by the subsequent occurrence of hæmaturia.

Secondary Lipoid Nephrosis.—In these cases there is a previous history of acute nephritis, with gradual failure of renal function, piling up of blood nitrogen, and death. This type of case is more common.

Biochemical Findings.—The urine.—Volume reduced, 20–30 oz. (600–900 c.c.). Sp. Gr., 1,020–1,025. Protein abundant, 10–60 g. per litre, chiefly albumin. NaCl diminished. *Cells*, leucocytes and epithelial cells, occasionally a red cell. *Casts*, hyaline, epithelial, granular and fatty.

The Blood.—Protein reduced to about 4%. This reduction

is due chiefly to loss of albumin, so that the globulin is relatively increased. In lipoid nephrosis, the protein may be 3·9%, of which albumin is 0·5% and globulin is 3·4%. Nitrogenous bodies, normal. Cholesterol, raised, 300 to 1,300 mg. per 100 c.c. The serum may have a milky appearance owing to the excess of lipoids.

Urea concentration test, normal figures.

Water elimination test, low figures.

Treatment.—On the supposition that the œdema is associated with the protein drain and lack of albumin in the blood, and also that it may have an extra-renal cause associated with perverted lipoid metabolism, Epstein (25) recommends treatment with a high protein and low fat diet. This should contain protein 120 to 240 g., carbohydrate 150 to 300 g., and fat 20 to 40 g. The total fluid should be between 40 and 50 oz. Venesection, followed by whole blood transfusion from a donor of a suitable group, is also useful in helping to restore the blood protein content to normal.

The table on p. 46 shows the diet which we use.

In addition, thyroid extract should be given in large doses (15 to 40 gr. daily), as long as the blood cholesterol remains raised.

In cases in which lipoid nephrosis is associated with nitrogen retention a high protein diet should not be given.

Amyloid Nephrosis.—This occurs in association with chronic pulmonary tuberculosis, chronic suppuration or syphilis. When in addition to the amyloid deposit in the kidneys lipoid nephrosis is also present, then œdema of the body results, the albuminuria increases, and the blood changes characteristic of nephrosis may be found. The treatment is that of the causative condition, and no special measures are required for the renal degeneration.

Chronic Diffuse Glomerulo-Nephritis.—This variety of nephritis is usually a sequel to an attack of acute nephritis in which albuminuria persists, and the patient suffers from varying degrees of œdema.

High Protein Diet

	Grams.			Calories.
	Protein.	Fat.	Carbo-hydrate.	
Breakfast—				
Fish (white), 6 oz.	30	0	0	120
Oatmeal (dry), 1 oz.	4·6	2	18	108
Ham (lean), 2 oz.	12	6	0	102
Milk (skimmed), 10 oz.	10	0	10	80
Bread, 2 oz.	4	0·8	28·6	138
Marmalade, ½ oz.	0	0	9	36
Tea, 5 oz.	0	0	0	0
Lunch—				
Meat (lean), 4 oz.	24	12	0	204
Peas or lentils, 2 oz.	4	0	8	48
Rice (dry), 1 oz.	1·6	0·1	22·7	98
Milk (skimmed), 10 oz.	10	0	10	80
Potato, 2 oz.	2	0	12	56
Tea—				
Bread, 2 oz.	4	0·8	28·6	138
Apple, 4 to 5 oz.	0	0	18	72
Tea, 5 oz.	0	0	0	0
Milk (skimmed), 1 oz.	1	0	1	8
Dinner—				
Fish (white), 4 oz.	20	0	0	80
Meat (lean), 4 oz.	24	12	0	204
Peas or beans, 2 oz.	4	0	8	48
Toast, 2 oz.	4	0	40	176
Rice (dry), 1 oz.	1·6	0·1	22·7	98
Milk (skimmed), 10 oz.	10	0	10	80
Coffee, 4 oz.	0	0	0	0
	170·8	33·8	246·6	1,974

Course.—This is usually prolonged, but progressive. Periodical attacks of acute nephritis may intervene, and gradually renal insufficiency sets in. There is a tendency to

generalised œdema, although this is variable. The heart gradually hypertrophies and the blood pressure rises. Retinal changes include hæmorrhages, exudates and œdema of the discs.

The œdema which is found early in the disease is probably of the acute nephritic type, later on it may be due in part to a superadded lipoid nephrosis, and in the final stages cardiac œdema may be present.

Biochemical Findings.—The Urine.—Volume, this may be diminished, normal or increased. Often the urine output is scanty when there is much œdema, and this is also true during the terminal stages. Sp. Gr. 1,020–1,026. Protein, present in fair amounts, as 5–10 g. per litre. Urea, diminished. *Cells*, epithelial cells, and occasional red and white blood corpuscles. *Casts*, hyaline and granular.

The Blood.—In the early stages there are no changes, later retention of urea and non-protein nitrogen or cholesterol occurs. Protein, normal.

Urea concentration test, low values in the later stages.

Water elimination test, low values in the later stages.

Treatment.—During the early stages, when there is œdema and only slight cardio-vascular changes, and little or no nitrogen retention in the blood, the treatment is as for subacute nephritis. Later, when the renal reserve power is lost, the treatment is as for chronic nephritis with slight or no œdema, of the malignant renal sclerosis type (see p. 49).

Chronic Nephritis with Slight or no Œdema

The practitioner is often called on to treat patients suffering from chronic nephritis without œdema (chronic interstitial nephritis). In such cases the blood pressure is generally raised, the heart enlarged and the urine contains a trace of albumin. It is important, especially from the point of view of prognosis and treatment, to decide whether the renal lesion is a benign type of nephritis in which renal

insufficiency is unlikely to develop, or whether the nephritis is malignant, with failure of kidney function. The methods of differentiating these conditions and their appropriate treatment will now be described.

Benign Renal Arteriosclerosis without Renal Insufficiency.—Clinical Findings. —The patient is usually a man between the ages of fifty and seventy. His symptoms are due largely to high blood pressure, myocardial weakness and arteriosclerosis. He complains chiefly of lassitude, headache, dyspnœa, and cardiac pain. A temporary paresis of one or more limbs or a transient aphasia may occur. The heart is hypertrophied and the blood pressure raised, the systolic reading often being about 200 mm. Hg. but the diastolic is not usually over 120 mm. Hg. Ophthalmoscopic examination may show retinal hæmorrhages, tortuous arteries crossing constricted veins, but a white star round the macula is not usually present.

Course.—This is progressive and death may occur from heart failure, coronary thrombosis, cerebral hæmorrhage, or some intercurrent infection. There is usually no renal failure.

Biochemical Findings.—The Urine:—Volume, normal or a little diminished. A relative increase in the night urine may occur. Sp. Gr. 1,005 to 1,012. Protein, a trace of albumin, as $\frac{1}{4}$ to 1 g. per litre. *Cells*, a few blood and epithelial cells. *Casts*, an occasional hyaline or granular one.

Blood.—Urea and non-protein nitrogen, normal or slightly raised if cardiac failure occur. Cholesterol, normal. Protein, normal.

Urea concentration test, normal.

Water elimination test, normal.

Treatment. —The treatment is that of cardio-vascular degeneration and high blood pressure, and no special treatment is required for the albuminuria.

Malignant Renal Sclerosis (of Fahr).—Clinical Findings.—The patient is usually an adult, frequently a male, between

the ages of forty and fifty. The onset is generally insidious, with frequency of micturition, especially by night, lassitude, anorexia, headache, giddiness and dyspnœa. More rarely there is an acute onset with cerebral hæmorrhage or uræmia. Cardio-vascular changes are present as hypertrophy of the heart, arteriosclerosis and increased blood pressure. The systolic pressure is often over 200 mm. Hg, and the diastolic between 120 and 160. Renal retinitis may occur with white lines radiating from the macula, exudates and hæmorrhages.

Course.—Renal sclerosis is progressive, the patient may live several years with increasing symptoms of chronic uræmia.

Biochemical Findings.—The Urine.—Volume increased, 80 to 100 oz. (2,400 to 3,000 c.c.), in the later stages it may be diminished, an equal quantity (as 30 c.c.) being passed every hour by day and by night. Sp. Gr. 1,002 to 1,008. Protein, $\frac{1}{2}$ to 5 g. per litre. *Cells*, renal epithelial cells and red cells from time to time. *Casts*, an occasional hyaline or granular cast.

Blood.—Urea and non-protein nitrogen raised, up to 100 mg. per 100 c.c. or over. Creatinin may be raised up to 2·5 mg. per 100 c.c. or over. Calcium may be low, 6 mg. per 100 c.c. or less. Cholesterol and Chlorides, normal.

Urea concentration test, low figures in the later stages.

Water elimination test, low figures.

Treatment.—This includes a general *régime*, with avoidance of chill, moderation in exercise, smoking, alcohol, and if possible a warm climate.

Diet.—The amount of protein allowed is determined approximately by the degree of nitrogen retention present. With moderate nitrogen retention (blood urea 50 to 80 mg. per 100 c.c.) a moderate protein diet (60 to 70 g. protein, is allowed (see p. 41). With high blood nitrogen figures (over 100 mg. urea per 100 c.c.) a low protein diet containing between 20 and 35 g. protein should be given.

Low Protein Diet

	Grams.			Calories.
	Protein.	Fat.	Carbo-hydrate.	
Breakfast				
Oatmeal, 1 oz. . .	4·6	2	18	108
Bread, 2 oz. . .	4	0·8	28	138
Butter, ½ oz. . .	0	12·5	0	112
Milk, 4 oz. . .	4	4	6	76
Tea, 4 oz. . . .	0	0	0	0
Lunch—				
Green vegetables, 2 oz.	0	0	2	8
Potato, 4 oz. . .	0	0	20	80
Bread, 2 oz. . .	4	0·8	28	138
Butter, ½ oz. . .	0	12·5	0	112
Stewed fruit (weighed raw), 2 oz. . .	0	0	8	32
Cream, ½ oz. . .	0·5	3	0·5	31
Jam, ½ oz. . . .	0	0	9	36
Rice, ½ oz. . . .	1	0	11	50
Tea—				
Bread, 1 oz. . .	2	0·4	14	69
Butter, ½ oz. . .	0	12·5	0	112
Tea, 4 oz. . . .	0	0	0	0
Jam, ½ oz. . . .	0	0	18	72
Milk, 2 oz. . . .	2	2	3	35
Dinner—				
Vegetable soup . .	—	—	—	—
Fish or meat or chicken, 2 oz. . . .	10	0	0	40
Potato, 4 oz. . .	0	0	20	80
Fruit, 4 oz. . .	0	0	16	64
Bread, 1 oz. . .	2	0·4	14	69
Sugar for sweetening, 2 oz. . . .	0	0	56	112
	34·1	50·9	271·5	1,574

The patient must be in bed while he is taking this diet, and it should not be persisted in for more than two to three weeks at a time, a return then being made to the moderate nitrogen diet. If the blood calcium be low, an endeavour should be made to restore it to a normal level by giving calcium lactate by mouth, gr. 15, t.d.s., and at the same time injecting Collip's parathormone, 10 to 20 units daily. This preparation is very potent and is put up in bottles containing 20 units in a c.c. Alternative methods for raising the calcium are intravenous injections of collosal calcium, 1 c.c. daily, or intramuscular injection of calcium chloride gr. 1, dissolved in 40 m. distilled water. The results must be gauged by blood analysis.

Uræmia in Chronic Nephritis.—Uræmic symptoms usually occur insidiously. Thus the appetite fails, a metallic or an ammoniacal taste may be noticed, and nausea, vomiting, constipation or diarrhœa may be troublesome. The headache is more persistent, the patient becomes drowsy and twitching of the muscles, hiccough or coma gradually ensue, with deep respirations at times of a hissing type. These are indications of irrevocable failure of renal function. More rarely uræmic amaurosis or coma develop suddenly, and then recovery for several years is possible in some cases.

Treatment for sudden coma or amaurosis consists in venesection or intravenous injection of hypertonic saline, as for convulsive uræmia (see p. 38). For the insidious stages of chronic uræmia, treatment is purely symptomatic. Saline purges may be given with the idea of removing intestinal toxins, but intractable diarrhœa results in some instances. Headache can be treated with bromides (gr. xv, t.d.s.), chloral hydrate (gr. v, t.d.s.), phenacetin (gr. v) and caffeine citrate (gr. iii) or morphine (gr. $\frac{1}{6}$). Muscular twitching may be relieved by intramuscular injections of calcium chloride gr. 1 in 40 m. distilled water. For gastric irritation, sedatives such as tinct. iodi m. 1 in ℥i water; chloretone gr. 5 and cocaine hydrochlorid. gr. $\frac{1}{5}$ as a powder in

water; acid hydrocyan. dil. m. 2; bismuth oxycarb. gr. 10; liq. adrenalin m. 5; or chloretone gr. 5, may be prescribed. Dyspnœa, if due to acidosis, may be relieved by sod. bicarb. ℥i, t.d.s., in water. It is always advisable for the alkali reserve to be estimated in uræmia, as this will reveal any acidosis, which may then be treated at once. Intractable hiccough is usually a terminal event and requires morphine.

Mixed Types of Chronic Nephritis

Many cases of chronic nephritis do not conform to type. Thus a patient may suffer from chronic nephritis with œdema, and yet have considerable nitrogen retention as well as excess of cholesterol in his blood. In such a case a high protein diet would be contra-indicated. In other instances, the blood cholesterol figures may be raised in chronic nephritis with slight or no œdema while the nitrogen figures approximate to normal. Here again, no advantage would be gained by a high protein diet. A similar patient may also pass blood in his urine from time to time, perhaps due to a flare up of a focal glomerulo-nephritic lesion, requiring treatment in bed as described on p. 38.

For the routine investigation and recording of a case of nephritis, the following nephritis case sheet may be of value :—

Nephritis Case Sheet

Name of patient
Age Sex M.S.W.
Family history of nephritis
Previous history
History of infectious fevers
History of pregnancy with albuminuria : Eclampsia.
No eclampsia.
History of present illness :—
Date of onset

Mode of onset. Acute—Hæmorrhagic.
Œdematous.
Toxæmic.
Insidious.

Frequency of micturition : Diurnal. Nocturnal.

Examination of patient.

General condition. Height
Weight (daily or weekly)

Heart : Size
Position
Sounds

Arteries

Blood pressure : Systolic. Diastolic.

Œdema : Legs Ascites
Arms Pleural effusion
Face Pericardial effusion
Sacrum Other situations
Lungs

Rashes

Uræmic manifestations : Nervous
Digestive
Respiratory

Secondary infections

Ophthalmoscopic examination

Blood count

Wassermann reaction

Basal metabolic rate

X-ray of renal tracts

Urea concentration test (date)
Urea 15 g. given at 8 a.m. (no breakfast taken)
Urea in grams per 100 c.c. urine : 9 a.m. 10 a.m.
11 a.m.

Water elimination test (date)
One pint of water drunk at 8 a.m. (no breakfast taken)
Urine passed at 9 a.m. 10 a.m. 11 a.m.

"Sterile" specimen of urine for bacteriological examination.

Examination of blood.

Date.	Per cent.				Milligrams per 100 c.c.						
	Total Solids.	Total Protein.	Alb.	Glob.	Urea.	N.P.N.	Sugar.	Cholesterol.	Ca.	Phosphates.	Cl as NaCl.
etc.											

Examination of urine.

Date.	8 a.m.—10 p.m.		10 p.m.—8 a.m.		Grams per litre.			Sugar. %	Blood.	Deposit.
	Vol. (c.c.).	Sp. gr.	Vol. (c.c.).	Sp. gr.	Total Protein.	Alb.	Glob.			
etc.										

REFERENCES

1. Paton.
   Brit. Med. Journ., 1890, ii., 197.
2. Hiller, McIntosh and Van Slyke.
   Journ. Clin. Invest., 1927, iv., 235.
3. Folin and Wu.
   See References Chapter XV.
4. Myers and Fine.
   Journ. Biol. Chem., 1913, xiv., 9–26.
5. Epstein.
   Journ. Amer. Med. Assocn., 1917, lxix., 444–467.
6. Epstein.
   Amer. Journ. Med. Sci., 1917, cliv., 638.
7. de Wesselow.
   Quart. Journ. Med., 1923, xvi., 341.
   Also "*Chemistry of the Blood*," 1924. Ernest Benn, Ltd., London.

8. Rowntree and Fitz.
Quoted in Barton's "*Manual of Vital Function Testing*," 1917. Badger, Boston.
9. Ambard.
Compt. Rend. Soc. Biol., 1910, 411.
10. Addis and Watanabe.
Journ. Biol. Chem., 1916, xxiv., 203.
11. Straus and Graunwald.
See 8.
12. McKaskay.
See 8.
13. MacLean.
"*Modern Methods in the Diagnosis and Treatment of Renal Disease*," 2nd ed., 1924. Constable & Co., Ltd., London.
14. Mosenthal.
Arch. Int. Med., 1915, xvi., 733.
15. Volcker and Joseph.
Münch. med. Woch., 1903, l., 2081.
16. Rowntree and Geraghty.
Journ. Pharm. and Exper. Therap., 1910, i., 579.
17. Berkeley, Dodds and Walker.
Journ. Obstet. and Gynæc. of Brit. Empire, 1924, xxxi., 20.
18. Andrewes.
Lancet, 1924, i., 590.
19. Harrison and Hewitt.
Brit. Med. Journ., 1927, ii., 1138.
20. Harrison and Bromfield.
Biochem. Journ., 1928, xxii., 43.
21. Hewitt.
Biochem. Journ., 1925, xix., 171.
Rabinowitch.
Canad. Med. Assocn. Journ., 1925, n.s., xv., 725.
Blotner and Fitz.
Journ. Amer. Med. Assocn., 1927, lxxxviii., 985.
22. Osman.
Guy's Hosp. Rep., 1927, lxxvii., 386.
23. Keith, Barrier and Whelan.
Journ. Amer. Med. Assocn., 1925, lxxxv., 799.
24. Müller.
Verhandl. der Deutsch. Path. Gesellsch., 1905, ix., 64.
25. Epstein.
Journ. Exp. Med., 1912, xvi., 719; *ibid.*, 1913, xvii., 444; *ibid.*, 1914, xx., 334.
Amer. Journ. Med. Sci., 1917, cliv., 638; *ibid.*, 1922, clxiii., 167.

CHAPTER III

GLYCOSURIA AND DIABETES MELLITUS

The history of diabetes mellitus, and of all conditions in which sugar appears in the urine, has been discussed so frequently that it would be superfluous even to summarise it here. Before one can obtain a clear insight into the pathology of glycosuria, it is necessary to know the outlines of carbohydrate metabolism.

The current belief is based on the work of Claude Bernard, although subsequent work has modified his views to some extent. It is believed that all carbohydrates are absorbed *viâ* the portal vein in the form of monosaccharides, mainly as glucose. On reaching the liver, glucose is polymerised into the polysaccharide glycogen, and is stored there, to be discharged into the blood stream as glucose. Just enough glycogen is broken down to keep the blood sugar more or less constantly at 100 mg. per 100 c.c. Thus the liver acts as a buffer between the gut and the systemic blood stream, and enables carbohydrate to be stored. The blood glucose is used up by the tissues, which oxidise it to CO_2 and water. In conditions where the intake of carbohydrate is normal, the liver can absorb sufficient glucose to prevent a marked raising of the blood sugar content, but according to the classical views, if an excessive amount be taken sugar appears in the urine and a condition of alimentary glycosuria results. The explanation of this lies in the fact that the liver has become saturated with glycogen, and the excess sugar has been passed straight into the systemic blood stream, constituting a condition of hyperglycæmia. The kidney responds

to this raising of the blood sugar above its normal limits, with the result that glucose appears in the urine. A normal kidney will excrete sugar if the concentration in the blood rise above 180 mg. per 100 c.c. This is known as the *leak point* of the kidney.

We have thus shown that the liver and kidneys play a very important part in the distribution of sugar throughout the body fluids, but we have not yet dealt with the utilisation of carbohydrates. It has been stated above that the blood sugar is used up by the tissues, and is oxidised to carbon dioxide and water, thus completing the metabolic chain. There is yet another controlling factor to be discussed, namely the bearing of the internal secretions upon carbohydrate metabolism. As is well known, the pancreatic internal secretion far outweighs the others in importance. Although Von Mering and Minkowski, in 1889, were the first to produce definite experimental evidence to show that pancreatectomy in dogs gives rise to a condition very similar to diabetes mellitus, the idea of associating the pancreas with glycosuria was of a much older date. Thus Conrad Brunner, in the seventeenth century, had attempted to prove a relationship; while Richard Bright, early in the nineteenth century, actually exhibited a specimen to show the relationship between pancreatic fibrosis and diabetes mellitus. Laguesse, following the work of Von Mering and Minkowski, showed that ligation of the pancreatic duct, although causing atrophy of the pancreas, did not produce glycosuria.

It became evident, therefore, that the anti-diabetic properties of the pancreas must lie in some function of internal secretion.

Opie was one of the first to suggest that this function might lie in the islets of Langerhans, and from the publication of this observer's works, the idea has gradually gained footing, until, at the present time, it has become almost universally accepted.

Again the history of the search for this anti-diabetic sub-

stance has been well recorded, and there is no need to do more than mention some of the attempts leading up to the discovery of insulin. Thus Rennie and Fraser, in 1905, prepared an active substance from the principal islets of certain teleostean fishes, whilst in 1908 Zuelzer obtained a pancreatic preparation with which he revived some comatose patients, reduced the glycosuria of depancreatised dogs, and benefited cases of diabetes mellitus.

These observations are all the more interesting since Zuelzer rendered his preparation fairly free from proteins by precipitation with alcohol. The research, however, was abandoned owing to the severe local reaction following injection. The culminating point in this long series of researches was reached in 1922, when Banting and Best (1) prepared a substance suitable for clinical use, which they called insulin. This substance was made by an alcoholic extract of the pancreas, and when injected subcutaneously, lowered the blood and urine sugar of depancreatised animals. The methods of preparation are very numerous, but practically all depend on extraction of the pancreas with alcohol. The alcoholic extract is evaporated *in vacuo* at 45° C. down to small bulk, when the fat separates out and can be removed. More alcohol is added to bring the percentage to about 80, when a precipitate of protein occurs, after which more alcohol is added in order to bring its concentration up to 92%, when insulin separates out.

This is a rough outline of Collip's (2) process, which has been modified by Doisy, Somogyi and Shaffer (3), using acidified alcohol, and by Dudley (4), using alkaline alcohol for the extraction.

The insulin so obtained must be purified, either by the method of precipitation at the iso-electric point, or by Dudley's (5) picrate method. Another method for the preparation was evolved by one of us (E. C. D.), working with F. Dickens (6), and consists in mincing the pancreas with solid picric acid, and extracting the insulin picrate with

acetone, from which it can be recovered by distilling off the acetone. The picrate is then converted to insulin hydrochloride by dissolving in acid alcohol and precipitating with acetone as in Dudley's method for the purification of insulin. This method gives greater yields than the alcohol processes, and is much more economical, both from the point of view of labour and materials.

Although it is definitely established that the injection of insulin profoundly modifies the metabolism of carbohydrates, very little is actually known as to the processes which bring about the reduction in blood sugar. The fall in blood sugar content commences immediately after the injection, and the degree of hypoglycæmia produced varies almost directly with the dose of insulin. The greater the dose, the more marked and rapid will be the fall in blood sugar content. When the amount of sugar in rabbit's blood reaches about 45 mg. per 100 c.c. the animal usually develops a peculiar convulsive condition, from which it can be revived by the intravenous injection of glucose. The study of the action of insulin resolves itself into an enquiry into the means by which this hypoglycæmia is produced. Considerable light can be thrown upon these problems by investigating the means by which hypoglycæmia can be relieved. These may be enumerated as follows :—

(1) By the injection of carbohydrates and their derivatives.
(2) By the injection of pituitrin ; and
(3) By the injection of adrenalin.

Glucose is the most efficient sugar, and only those sugars readily fermented by yeast (glucose, fructose and mannose) possess the property of relieving hypoglycæmia. The injection of pituitrin was shown by Burn (7) to inhibit the action of insulin, and similar results were found when adrenalin was injected into a hypoglycæmic animal. The exact manner in which insulin operates can only be surmised by reviewing

the possibilities, and by adopting an eliminative argument. These possibilities can be summarised as follows :—

I. The injection of insulin may activate the blood in some manner which causes it to destroy glucose.

II. The effect may be produced by stimulating the glycogenetic functions of the liver, which would thus remove glucose from the blood stream and store it as glycogen.

III. Insulin might stimulate the oxidative processes of the body, with the result that glucose is removed at an increased rate.

IV. Insulin might cause the sugar to be converted into an unknown non-reducing substance, which would be stored in the body.

The first three possibilities have been excluded by a vast amount of experimental work. Thus the Toronto workers showed that insulin had no effect on *in vitro* glycolysis. All workers have proved that if insulin be given to a normal animal, its store of glycogen is depleted when hypoglycæmia supervenes. If, however, insulin be injected into a diabetic animal, glycogen storage is at first increased, since insulin restores it to the normal condition. If more insulin be given and hypoglycæmia be produced, the glycogen store will be reduced as in the normal animal. Although there is an increase in the respiratory metabolism following injection of insulin, the increased oxygen consumption is not sufficient to account for the disappearance of the glucose. We are driven, therefore, to the somewhat unsatisfactory theory that the glucose is converted into some unknown, non-reducing substance. Recent work by Dale and his co-workers shows that the action of insulin may be explained by an increased glycogen formation, together with an increase in the oxidation of glucose. The theories of Winter and Smith with regard to the nature of the blood sugar have not received confirmation, and since so much doubt seems to surround this

problem, they will not be discussed here. Before leaving this subject it will not be out of place to refer to two papers, the results of which seem to require explanation. Best, Smith and Scott (8) stated that they were able to recover insulin from the organs of a diabetic dog which had been depancreatised some weeks beforehand. An exactly parallel observation was made by one of us (E. C. D.) working with S. L. Baker and F. Dickens (9). We found that the pancreas, muscle, liver, etc., of a patient who had died from diabetic coma contained relatively large quantities of insulin. It would appear, therefore, that diabetes mellitus can occur when there is insulin in the body, but the true interpretation of these independent observations has yet to be put forward.

For a full account of these problems the reader is referred to the book by F. Dickens and one of us (E. C. D.), and to other publications referred to under reference No. (10).

Insulin, then, is a necessary factor in the metabolism of sugar. The internal secretions of the thyroid, pituitary and suprarenals also affect sugar metabolism, although not to the same extent as insulin. Finally, there is the nervous influence on carbohydrate metabolism. Thus Claude Bernard was able to produce glycosuria by puncturing the floor of the fourth ventricle. This glycosuria is of a temporary nature, but is constantly observed experimentally. Its explanation will be dealt with later.

We see, therefore, that there are the following factors influencing carbohydrate metabolism :—

1. The intake and absorption of carbohydrate.
2. The liver.
3. The kidneys.
4. The pancreas.
5. Certain other glands, such as thyroid, pituitary and suprarenals.
6. The nervous factor.

Failure or variation of any of these factors may produce

glycosuria, and it will, perhaps, be as well to summarise briefly the mechanism of each.

Alimentary Glycosuria

This is due, as already explained, to overloading the liver with sugar, with the result that the storage capacity is exhausted, and the consequent increase in blood sugar is compensated by excretion into the urine. Normally 100 g. of glucose should not produce glycosuria ; that is to say, the physiological individual can tolerate 100 g. of glucose in one dose, and can store it. Should sugar appear in the urine after such a quantity, the patient's tolerance is said to be below normal, and some disturbance of carbohydrate metabolism is indicated. This forms a clinical test, although it is open to many fallacies. Obviously, some account should be taken of the patient's height and weight when working out the tolerance.

The above statements represent what might be termed the classical view. In 1916 Taylor and Hulton (11) stated they were unable to induce alimentary glycosuria by the ingestion of large amounts of glucose. MacLean (12), in 1924, also cast some doubt upon the ease with which this condition could be produced. Working with T. Izod Bennett, one of us (E. C. D.) (13) repeated these experiments, and found that even 500 g. of glucose B.P. were unable to produce glycosuria in the majority of normal men, and it was concluded that the upper limits of carbohydrate tolerance are governed merely by the subject's appetite, and that any attempts to demonstrate increased carbohydrate tolerance by alimentary tests are useless.

Hepatic Glycosuria

Since the liver plays such an important part in the metabolism of carbohydrates, it would be expected that disorders

of this organ might be attended with the appearance of sugar in the urine. Such an event, however, is rarely seen in any of the hepatic diseases. Cammidge (14) holds that certain forms of glycosuria are of hepatic origin, and he describes tests which are said to distinguish these cases from those of true pancreatic diabetes. Many authorities do not agree with Cammidge, and hold that all forms of progressive diabetes are of pancreatic origin.

The reader should refer to the works of Cammidge given in the bibliography for this author's views.

Renal Glycosuria

The normal kidney will not let sugar pass into the urine unless the concentration in the blood is over 180 mg. per 100 c.c. This is known as the leak point of the kidney. In the condition under discussion, the leak point is lower than the normal, hence sugar escapes into the urine although the blood sugar is below 180 mg. per 100 c.c. There are varying degrees of renal glycosuria, according to how far the leak point is removed from the normal. If only a little below, say 140 mg. per 100 c.c., the sugar will only appear in the urine after carbohydrate meals, when the blood sugar is likely to rise.

In more severe cases the urine will always contain sugar. As can be seen, this condition is characterised by glycosuria in the presence of a low or normal blood sugar. Renal glycosuria is usually regarded as an innocent condition requiring no treatment, but it is possible that in some cases it may be followed by the development of a real diabetes. For this reason it is advisable for blood sugar tolerance estimations to be made from time to time.

Pancreatic and Endocrine Glycosuria

Here the condition is due to the absence of the internal secretion of the pancreas, which enables the tissues to utilise

glucose. The traditional explanation of the pathology of diabetes mellitus is that the islets of Langerhans fail to produce the anti-diabetic hormone, with the result that the tissues cannot metabolise glucose. That diabetes mellitus is associated with definite lesions of the islets of Langerhans has been demonstrated by many workers. A great deal of histological work has been done upon these bodies, and minute details of the histological lesions in diabetes mellitus have been published. According to Bensley and Lane (15), the cells found in the islets are of two varieties, α and β according to whether their granules are fixed respectively by alcoholic or aqueous media. The β cells are stated to be affected in diabetes mellitus, since they are seen to contain no granules. According to this school, diabetes mellitus is a disease of the β cells of the islets of Langerhans. This inability to utilise carbohydrate is interpreted by the body as a stimulus for the production of more glucose, with the result that all available carbohydrate is poured out into the blood, producing a hyperglycæmia, and, since the tissues cannot utilise the sugar, it is excreted in the urine.

Although the above explanation can be accepted as a working clinical hypothesis, recent research on the pharmacological action of insulin tends to show that the pancreas plays a much more important and complicated *rôle*.

When all the available carbohydrate has been thrown into the circulation, some proteins are converted into glucose and are excreted. The fat metabolism is also affected, producing a condition of acidosis or ketosis.

In hyperthyroidism, glycosuria is fairly common, whilst in certain pituitary lesions it is also observed.

An injection of adrenalin also gives rise to glycosuria. The action of this hormone is said to lie in the fact that it stimulates the liver to break down glycogen, with the result that hyperglycæmia and glycosuria ensue. Adrenalin, therefore, appears to antagonise the action of insulin, which tends to inhibit the breaking down of glycogen. It is

known experimentally that adrenalin will help to abolish insulin convulsions. Pituitrin also inhibits the action of insulin.

Nervous Influences. Diabetic piqûre

By stabbing the floor of the fourth ventricle, sugar can be made to appear in the urine, and this is preceded by hyperglycæmia. This effect cannot be produced if the splanchnic nerves be cut. The explanation is probably that the diabetic puncture causes a stimulus to the suprarenals *viâ* the cord and splanchnic nerves, with the result that adrenalin is discharged into the circulation. This causes the hepatic glycogen to be broken down to glucose, as in the case of adrenalin glycosuria. Although this form of glycosuria is mainly of theoretical interest, cases occur where pressure of cerebral tumours, a cerebral hæmorrhage or meningitis, etc., cause the appearance of sugar in the urine.

Chemical Examinations Necessary in Cases of Suspected Glycosuria

Examination of the Urine

Qualitative Tests for Sugar

All the common tests for sugar depend on the reduction of an alkaline copper solution on boiling with the suspected solution. *Fehling's test* is the best known, and, at the same time, one of the most misleading and inaccurate tests, both qualitatively and quantitatively. It is so well known that it will not be described.

Fehling's reagent is reduced by a number of other substances, such as uric acid, creatinin, glycuronic acid, lactose, pentose, and homogentisic acid. Further, owing to the strong alkali present, small quantities of sugar may be destroyed on boiling as the result of conversion into caramel. Hence a positive reaction to Fehling's test does not necessarily

mean that the patient is suffering from glycosuria, nor, on the other hand, does a negative reaction exclude the presence of small traces of glucose. We see, therefore, that Fehling's test is thoroughly unsuitable for clinical work.

Fortunately we have another test equally simple, yet much more accurate. *Benedict's test*[1] overcomes most of the errors of Fehling's reaction.

To carry out the test, 5 c.c. of the reagent are poured into a test tube, and 8 drops of urine are added. The contents of the tube are boiled over the flame for two minutes and then allowed to cool. If a large number of tests are to be made, the tubes may be set up in a rack and immersed in a boiling water bath for five minutes. The technique described above must be rigidly adhered to, since the actual quantities of urine, reagent, and time of heating were all worked out after a careful series of experiments. Any departure from it will rob the test of its value, namely its specificity. If glucose be present, the reagent will change colour from clear blue to an opalescent green, or, if a large quantity of sugar be present in the urine, the final colour will be an opaque red. It is possible to obtain some idea of the amount of sugar present in the specimen by a study of the colour of the contents of the test tube. The following scheme will indicate the kind of reductions given by urines containing quantities of sugar between 0·1% and 2% :

0·1% greenish opalescence ; no red colour ; no precipitate.

0·2% as above, but slight yellowish precipitate ; fluid blue on standing.

0·3% green colour, with slight orange precipitate ; fluid blue on standing.

[1] The qualitative reagent is prepared as follows :—

One hundred and seventy-three g. of crystallised sodium citrate and 100 g. of anhydrous sodium carbonate (or 200 g. crystallised) are dissolved in 700 c.c. of water. 17·3 g. of fine crystallised copper sulphate are dissolved in 100 c.c. of water. The copper sulphate solution is poured into the former solution, and the volume is made up to 1,000 c.c.

0·5% definite orange precipitate; fluid still blue on standing.

1·0% heavy orange-brown precipitate; still some blue colour in fluid on standing.

2·0% bright red, heavy precipitate; blue colour of fluid almost disappeared.

Benedict's test possesses the advantage of being standard, that is to say, 5 c.c. of reagent are tested with 8 drops of urine, and the boiling time is kept constant. There are no hard and fast conditions with Fehling's test, hence the results obtained by different workers are not comparable. Benedict's reagent is reduced by glucose, lævulose, lactose, pentose, and homogentisic acid, but not by uric acid and creatinin; hence a positive Benedict's test implies some derangement of carbohydrate metabolism. In the majority of cases a positive Benedict's test means glycosuria, yet the possibility of lactosuria and pentosuria should never be lost sight of. The former condition occurs in pregnant and lactating women, whilst the latter is a rare disorder of metabolism, to some extent simulating diabetes mellitus. There are many ways of identifying what particular sugar is causing the reduction. Thus some workers prefer to prepare osazones and identify the sugar by the microscopic appearance of its phenylhydrazone, whilst pentoses can be differentiated from other urinary sugars by observing what deflection is caused on the plane of polarised light and by special tests, such as that of Bial.[1] Glucose and lactose are dextro-rotatory, whilst urinary pentoses are without action on polarised light. These methods, although accurate, require apparatus and special skill, and hence are unsuited to general practice. The best reaction for clinical purposes is the fermentation test. A little yeast is ground up with the suspected urine until a thin paste is formed, and

[1] *Bial's test for pentose.* 5 c.c. of Bial's reagent (1·5 g. orcinol, 500 g. fuming HCl, 20–30 drops of 10% $FeCl_3$ solution) are boiled in a test tube, and 2–3 c.c. of urine are added. The heating is discontinued, and if pentoses be present, the solution turns green in colour.

a fermentation tube is filled with the mixture. A urine definitely known not to contain sugar and one to which glucose has been added are treated similarly, and the three tubes are set up in a warm place overnight. In the morning they are inspected, and, if sugar be present in the suspected urine, the top of the fermentation tube will contain gas, as will also the tube containing the positive control. There must be no gas in the tube containing the urine with no sugar. It must be emphasised that the positive and negative controls form part of the test, and must never be omitted, since it is possible to have a yeast that will not ferment glucose, or on the other hand, one which will produce gas when no glucose is present.

Having ascertained the presence of glucose with certainty, one proceeds to the tests for acetone bodies.

It must be thoroughly realised that these tests are of much greater importance than the percentage of sugar, since the presence of acetone bodies in large amounts means that the patient is in imminent danger of coma. Every patient whose urine contains large quantities of these bodies should be kept under close observation.

Tests for Acetone Bodies

Aceto-acetic Acid

Gerhardt's Test.—This test consists in adding 10% ferric chloride, drop by drop, to the urine. At first a precipitate of ferric phosphate appears, which usually redissolves in excess of the reagent, owing to its acidity. If aceto-acetic acid be present in concentration of 0·07% or over, the solution turns a Bordeaux red colour. If any doubt be felt upon the genuineness of the colour, it should be compared with that given by ferric chloride added to normal urine. If a positive reaction by this test be given, an alteration must be made in the treatment, since a severe degree of ketosis is preseut. On the other hand, a negative reaction

does not signify that the patient is entirely free from the dangers of ketosis. The reason for this is two-fold : firstly, the relative insensibility of the reaction ; and secondly, the fact that urinary aceto-acetic acid is converted on standing into acetone, which does not give Gerhardt's test.

Gerhardt's test is not without fallacies. Thus the presence of any phenolic drug in the urine will give a colour reaction with ferric chloride somewhat similar to that obtained with aceto-acetic acid.

If the patient has been taking drugs, such as aspirin, salicylic acid or salicylates and their derivatives, misleading results may be obtained. The colour, however, is quite different from that given by aceto-acetic acid, since it is much darker and of a more violet hue. Although this difference can be easily detected with practice, it is a good plan to boil a specimen of the urine prior to the test. If the colour be due to aceto-acetic acid, a negative reaction will be given on the addition of ferric chloride, whilst in the case of phenyl derivatives the colour will not be affected. This, of course, is due to the fact that aceto-acetic acid is volatile, whilst the other compounds are not.

Rothera's Test for Acetone and Aceto-acetic Acid.[1]—This reaction is by far the most satisfactory test for ketosis. It is carried out by saturating about 20 c.c. of urine with ammonium sulphate, by shaking with the crystals in a test tube. Two or three drops of concentrated ammonia, and a few drops of a freshly-prepared dilute solution of sodium nitroprusside in water, are added, and the tube is shaken.

If the reaction be positive, a delicate permanganate tinge develops, which gradually deepens. A brown colour does not constitute a positive reaction. The amount of aceto-acetic acid can be judged by the depth of colour and the rapidity with which it develops. Thus Kenna-

[1] It is essential that this test be performed in a laboratory that is not used for blood analysis, otherwise contamination of the nitrogen-free blood reagents will occur and inaccuracies will result.

way (16) points out that Rothera's test can be classified as follows :

1. A quick, strong reaction corresponding to 0·25% aceto-acetic acid.

2. A slow, weak reaction corresponding to 0·0005% aceto-acetic acid.

Reactions intermediate between these two extremes indicate proportional concentrations of aceto-acetic acid.

A faintly positive Rothera's test is not of such grave importance as a positive Gerhardt's reaction, owing to the much greater delicacy of the former.

There are no fallacies in Rothera's test, and it should be performed on every diabetic urine.

Quantitative Tests on Diabetic Urine

Estimation of Sugar

There are two classes of methods available—volumetric and polarimetric.

Volumetric Methods

Practically all these depend upon the reduction of an alkaline copper solution. Some, such as Fehling's, are inaccurate ; others, such as Bertrand's or Benedict's methods, are tolerably accurate. Fehling's quantitative process gives very inaccurate results, and the end point is very hard to recognise, whilst Benedict's method will be found to be satisfactory for general use. Since the techniques for Fehling's and Benedict's tests are similar, they will be described together.

Benedict's Quantitative Solution.—The following are dissolved by heating in enough water to make 800 c.c. :—

Sodium citrate . . .	200 g.
Sodium carbonate (crys.) . .	200 g.
Potassium thiocyanate . .	125 g.

18·0 g. of pure copper sulphate (air dried) are dissolved in 100 c.c. of water, and the resulting solution is poured slowly into the first. Five c.c. of 5% solution of potassium ferrocyanide are added, and the volume is made up to 1,000 c.c.; 25 c.c. of this reagent (corresponding to 0·05 g. of glucose) are pipetted into a small flask.

Three to 4 g. of anhydrous sodium carbonate are added, and the whole is brought to the boil. Urine is then run in from a burette slowly until the reagent turns from clear blue to an opalescent bluish-white colour, when the additions are made more carefully until the colour disappears. This preliminary titration gives an idea of the concentration of sugar in the urine, but for accurate results another titration must be performed. Working on the knowledge gained in the previous experiment, the urine is diluted so that about 10 c.c. will reduce the reagent, and the titration is performed again. The following is a typical example :—

First Titration : One c.c. of urine reduced the reagent, hence this quantity of urine must have contained 0·05 g. of glucose, *i.e.*, 5%. Obviously the result cannot be very accurate when only 1 c.c. of urine was run in from a burette. It was therefore diluted 1 in 10, and the titration performed again.

Second Titration : 9·5 c.c. of the diluted urine just reduced the reagent, hence 0·95 c.c. of undiluted urine contained 0·05 g. sugar. The percentage was therefore $\frac{100 \times 0{\cdot}05}{0{\cdot}95}$ = 5·26%.

The most important part in the method is to run the urine into the boiling reagent at the same rate every time the test is performed. Any error is then constant throughout a series of titrations.

With Fehling's method exactly the same technique is adopted, except that 10 c.c. of the reagent are taken and diluted to 25 c.c. Urine is run into the boiling mixture until

the blue colour has disappeared. The end point is very hard to recognise, and the same objections arise as in the qualitative test. Ten c.c. of Fehling's reagent are equivalent to 0·05 g. of glucose.

Polarimetric Methods

These are suitable for hospital work, where large numbers of estimations have to be performed. The methods are very accurate, and most of the objections are mainly theoretical. Thus, since certain bodies, such as β-oxy-butyric acid, are lævo-rotatory, they would tend to reduce the dextro-rotatory power of the urine. The quantity of these bodies is, however, very small, and their specific index of rotation is negligible when compared to the dextro-rotatory powers of glucose. Before taking a polarimetric observation, it is usual to clarify the urine by some procedure. If 10 c.c. of 10% basic lead acetate solution be added to 40 c.c. of urine, the filtrate obtained after shaking is quite suitable for observation. Very useful and cheap saccharimeters are made by the Zeiss and Reichert Companies. These instruments can be used with daylight, and the readings on the scale give the percentage of sugar in the urine.

Table showing the Excretion of Sugar in various Representative Cases.

Case.	Sugar. %	Volume of urine in 24 hours. c.c.	Total amount of sugar in 24 hours. g.
Mild diabetes mellitus .	0·5	1,800	9
Severe diabetes mellitus .	9	5,500	495
Renal glycosuria . .	0·2	1,200	2·4
Thyroid glycosuria . .	1	1,300	13·2

Estimation of Total Acetone Bodies (Van Slyke (17))

Solutions.—1. 10% mercuric sulphate solution. 73 g. of pure red mercuric oxide are dissolved in 1 litre of $4N.H_2SO_4$.

2. 20% copper sulphate solution. 200 g. of $CuSO_4.5H_2O$ are dissolved in water and made up to 1 litre.

3. 10% calcium hydroxide suspension. 100 g. of Merck's fine light "reagent" $Ca(OH)_2$ are mixed with 1 litre of water.

4. 50% sulphuric acid (by volume). 500 c.c. of sulphuric acid (sp. gr. 1·835) are diluted to 1 litre with water. The concentration of H_2SO_4 must be readjusted if necessary to make it 17 N. by titration.

5. 5% potassium dichromate. 50 g. of $K_2Cr_2O_7$ are dissolved in water and made up to 1 litre.

Combined reagents for total acetone bodies determination :

1 litre of the above 50% H_2SO_4.
3·5 litres of the mercuric sulphate.
10 litres of distilled water.

Method.—Before starting the determination, glucose and other interfering substances must be removed. Measure 25 c.c. of urine into a 250 c.c. volumetric flask, add 100 c.c. of water, 50 c.c. of the copper sulphate solution and mix. Then add 50 c.c. of 10% calcium hydroxide, shake, and test with litmus ; if not alkaline, add more calcium hydroxide, dilute to the mark with water and leave standing for at least half an hour for glucose to precipitate and then filter. This process will remove up to 8% glucose ; urine containing more should be diluted to bring glucose down to this figure. The copper treatment must not be omitted ; it removes other interfering substances besides glucose.

Boil a small quantity of the filtrate in a test tube ; if the removal has not been complete, a precipitate of yellow cuprous oxide will be obtained. A slight precipitate of white calcium salts always forms, but this does not interfere with the detection of the yellow cuprous oxide.

To determine simultaneously the total acetone bodies (acetone, aceto-acetic acid, and hydroxybutyric acid) in one operation, proceed as follows :—

Place 25 c.c. of the urine filtrate, 100 c.c. of water, 10 c.c. of 50% H_2SO_4, and 35 c.c. of 10% mercuric sulphate in a 500 c.c. conical flask. Instead of adding the water and reagents separately, 145 c.c. of the " combined reagents " may be added.

Connect the flask with a reflux condenser, having a straight condensing tube of 8–10 mm. diameter, and heat to boiling. After boiling has commenced, add 5 c.c. of the 5% dichromate through the condenser tube. Boil gently for one and a half hours. The yellow precipitate which forms consists of the mercury sulphate-chromate compound ($3HgSO_4.5HgO.\ 2(CH_3)_2CO$) of the preformed acetone, and of the acetone which has been formed by decomposition of aceto-acetic acid and by oxidation of the hydroxybutyric acid.

It is collected in a Gooch or " medium density " alundum crucible, washed with 200 c.c. of cold water, and dried for an hour at 110° C. The crucible is allowed to cool in room air and weighed (a desiccator is unnecessary and undesirable). Several precipitates may be collected, one above the other, without cleaning the crucible.

Calculation.—Since in the first operation the urine is diluted 1 in 10, the 25 c.c. of filtrate employed are equivalent to 2·5 c.c. of undiluted urine. One mg. of acetone yields 20 mg. of precipitate. For the calculation the following formula may be used :—

$$\frac{\text{mg. of precipitate}}{20} \times \frac{\text{vol. of urine}}{2{\cdot}5} = \text{mg. of total acetone bodies in specimen, calculated as acetone.}$$

Blood Examinations in Cases of Glycosuria

Having performed the examinations detailed above, we now proceed to an examination of the blood sugar.

A very good plan is to perform a sugar tolerance test, using the estimation of blood sugar as a criterion. The alimentary method detailed on p. 62 has been almost superseded by this form of test. The patient comes to the laboratory in the morning starving from the night before. Blood is collected and the patient is given 50 g. of glucose, dissolved in 300 to 400 c.c. of water, to drink. Blood is collected at half-hourly intervals for two hours, thus making four specimens after the glucose and one before. The sugar content of each is estimated by any one of the methods described in the chapter on blood analysis.

The following table gives typical findings in various conditions. These findings are taken from the records of the Courtauld Institute, Middlesex Hospital.

Blood Sugar Content in mg. per 100 *c.c.*

Case.	Before Glucose.	½ hour after.	1 hour after.	1½ hours after.	2 hours after.
Normal man .	100	120	130	110	100
" Lag " Curve .	100	150	230	140	120
Mild diabetes .	170	187	198	190	182
Severe diabetes .	240	270	294	300	314
Renal glycosuria	98	100	94	96	98

Normally there is a rise in sugar content following the meal, but never above 150 mg. per 100 c.c. The sugar content is back to normal again in two hours. In some cases the blood sugar rises sharply to above 180 mg. per 100 c.c., but returns to normal within the two hours. This is called a " lag " curve, and indicates that the sugar-storage mechanism cannot keep pace with the glucose absorption, and there is therefore a storage lag indicated by the rapid rise above the upper normal limit. This is regarded as one of the first stages in the production of a true diabetes mellitus. In the mild dia-

betic the resting blood sugar is higher, and after the administration of glucose the content rises above the leak point of the kidney, 180 mg. per 100 c.c., and the return to normal is not so sharp. In the severe diabetic this condition is accentuated, and there may be no attempt at a return to the previous level during the period of the test.

Renal glycosuria is characterised by a subnormal blood sugar content throughout the experiment. This method, therefore, is extremely useful in diagnosing the type of glycosuria, and, should it prove to be diabetes mellitus, in judging the gravity of the condition.

Ketosis or Acidosis in Diabetes Mellitus

Up to the present the only reference to this condition has been the description of the tests for acetone bodies in the urine. These are by far the most important, and, owing to their simplicity and accuracy, must remain as the most suitable clinical tests for this condition.

The whole question of ketosis is intimately bound up with that of acidosis ; consequently it is necessary to enter into the physiology of neutrality regulation. Space does not allow of a full treatment of this subject.

Firstly, it is assumed that the acidity of a solution depends upon the relative preponderance of hydrogen ions over hydroxyl ions. From the point of view of reaction, the product of the H and OH ions, expressed as gram ions per litre, is always constant ; hence, if the H ions increase, then the OH ions diminish in such proportions as to make the product of the two constant. By various physico-chemical means, it has been ascertained that $[H] \times [OH] = K = 10^{-14}$ when expressed as gram ions per litre.

Now, at absolute neutrality, the H ion concentration is such that it equals the OH ion concentration.

Expressed as an equation, we have the following state of affairs at absolute neutrality.

$$[H] \times [OH] = K = 10^{-14},$$

but $[H] = [OH]$,

i.e., $[H] = 10^{-7}$ and $[OH] = 10^{-7}$.

The important point to realise is that there is a definite hydrogen ion concentration at absolute neutrality, but that it is of such a figure that it equals the OH ion concentration, *i.e.*, 10^{-7}. The majority of students possess the erroneous idea that absolute neutrality is the point at which the H ion concentration sinks to zero.

Acid solutions have H ion concentrations, or C_H as it is called, above 10^{-7}, *i.e.*, 10^{-6}, 10^{-5}, etc., whilst the reverse holds good for alkaline solutions.

Although the C_H nomenclature mentioned above is occasionally used, it has been almost entirely superseded by the logarithmic nomenclature of Sörensen. Here the hydrogen ion concentration is designated by the symbol *p*H, or the logarithmic exponent.

For those familiar with logarithms, the following explanation will be quite clear :—

Let us work out the *p*H of absolute neutrality.

$C_H = 10^{-7}$.

$C_H = 1{\cdot}0 \times 10^{-7}$.

*p*H = index of 10 in above equation (omitting − sign) − log. of figure expressing normality.

i.e., *p*H $= 7 -$ log. 1,

*p*H $= 7$.

All *p*H are calculated from the C_H in this manner. Thus the C_H is expressed as 10 to a negative index multiplied by a number with one figure to the left of the decimal place, this representing the normality. Owing to the minus sign, it can be seen that *p*H will vary inversely as C_H, or, that is to say, any *p*H above 7 will denote an alkaline solution, any below 7 an acid solution. The advantages of this nomenclature are considerable, one of them being that the hydrogen ion concentration is expressed as one figure instead of 10 to a

negative index. Below will be found the *p*H of some common fluids.

	*p*H
Neutral distilled water. . .	7·0
$\frac{N}{10}$ acid	1·0
$\frac{N}{10}$ alkali	13·0
Blood	7·45
Urine	5 to 8

There are many methods for the determination of hydrogen ions, but, from the standpoint of this book, the indicator method alone need be described. The properties of litmus, perhaps, are the most familiar in this respect.

In acid solution this indicator turns red, whilst in alkaline solution the colour changes to blue. This colour change is due entirely to the concentration of H ions, and acts by dissociating or associating organic acids and bases in the indicator. The change in colour of any particular indicator takes place at a constant *p*H, and, since this is known for most indicators, a ready means for the estimations of *p*H is provided.

The colour changes and *p*H of the principal indicators are given below.

Indicator.	*p*H	*Colour.*	
		Acid.	*Alkaline.*
Hæmatoxylin . . .	0·0 — 1·0	Pink	— Greenish.
Phenol-tetra-brom-phthalein	0·3 — 8·0	Colourless	— Violet.
Iodo-Eosin . . .	0·3 — 14·0	Green-Yellow	— Pink.
m-Cresol Purple . .	0·5 — 2·5	Red	— Yellow.
Metanil Yellow . .	1·2 — 2·3	Red	— Yellow.
Thymol-Blue . . .	1·2 — 2·8	Red	— Yellow.
Tropœolin OO (Orange IV.)	1·4 — 2·6	Pink	— Yellow.
Brom-Phenol Blue . .	2·8 — 4·6	Yellow	— Blue.
Methyl-Orange. . .	2·9 — 4·0	Red	— Yellow.
Di-methyl-amino-azo-benzene	2·9 — 4·0	Crimson	— Yellow.
Congo-Red . . .	3·0 — 5·0	Blue	— Scarlet.
Benzene-azo-naphthylamine	3·7 — 5·0	Pink	— Yellow.
Brom Cresol Green . .	4·0 — 5·6	Yellow	— Blue-Green.

Indicator.	*p*H	*Colour.*	
		Acid.	*Alkaline.*
Alizarin Red S.	4·0 — 6·0	Yellow-Green	— Red.
Lacmoid	4·0 — 6·0	Pink	— Violet-Blue.
a-Naphthylamine-azo-benzene-*p*-sulphonic acid	4·2 — 5·8	Pink	— Yellow.
Methyl Red	4·4 — 6·0	Red	— Yellow.
Diethyl Red	4·5 — 6·5	Pink	— Yellow.
Propyl Red	4·5 — 6·5	Pink	— Yellow.
Phenacetolin	5·0 — 6·0	Brown-Red	— Pink.
Cochineal	5·0 — 6·0	Brown-Pink	— Lilac.
p-Nitro-phenol	5·0 — 7·0	Colourless	— Yellow.
Azo-Litmin	5·0 — 8·0	Red	— Blue.
Brom Cresol Purple	5·2 — 6·8	Yellow	— Purple.
Brom Thymol Blue	6·0 — 7·6	Yellow	— Blue.
Brilliant Yellow	6·4 — 9·4	Yellow	— Orange.
Neutral Red	6·8 — 8·0	Red	— Yellow.
Phenol Red	6·8 — 8·4	Yellow	— Red.
Rosolic Acid	6·9 — 8·0	Brown	— Red.
Tropœolin OOO	7·0 — 8·9	Yellow	— Pink.
Cresol Red	7·2 — 8·8	Yellow	— Red.
a-Naphthol-sulphone-phthalein	7·5 — 9·0	Yellow	— Blue.
m-Cresol Purple	7·6 — 9·2	Yellow	— Purple.
a-Naphthol phthalein	7·3 — 8·7	Red	— Blue.
Thymol-Blue	8·0 — 9·6	Yellow	— Blue.
Phenol-tetra-chlor-phthalein	8·2 — 9·4	Colourless	— Violet.
o-Cresol-phthalein	8·2 — 9·8	Yellow	— Violet.
Phenol-thymol phthalein	8·3 — 11·0	Colourless	— Violet.
Phenol phthalein	8·3 — 10·0	Colourless	— Pink.
Alizarin Blue S.	9·0 — 13·0	Green	— Blue.
Thymol Phthalein	9·3 — 10·5	Colourless	— Blue.
Alizarin Yellow G	10·1 — 12·1	Yellow	— Red.
Alizarin	11·0 — 13·0	Lilac	— Blue.
Porrier's Blue	11·0 — 13·0	Blue	— Red.
Tropœolin O (Resorcin Yellow)	11·1 — 12·7	Yellow	— Red-Orange.

The *p*H of the urine may be determined by this method. There is on the market a "Universal indicator" (British Drug Houses), composed of a series of those indicators already quoted. If a drop of this be added to the urine a certain colour develops, and by reference to the table on the bottle, the *p*H may be read off against the colour. This is, of course, only a rough method.

These methods are sufficiently accurate for clinical work, but for the more accurate investigations it is usual to adopt an electrometric method.

The hydrogen ion concentration of the blood under physiological conditions is always kept constant. It is only in the terminal stages of disease that any alteration occurs, and this usually precedes death. The hydrogen ion concentration of blood is perhaps one of the most constant things in Nature. That this is so becomes all the more surprising when one considers the number of factors which are tending to alter it.

Thus there is the constant production of acid from metabolic processes and muscular action. Again, large quantities of acid are poured off the blood during gastric secretion, and large quantities of alkali during pancreatic secretion. It is obvious that the regulating mechanism must be wonderfully efficient to keep the reaction of the blood constant during gastric secretion, since the HCl secreted must be derived, in the first place, from the blood.

The acid-base equilibrium system is a very complex one, employing, as it does, practically every system in the body. The various factors for keeping the reaction of the blood constant are summarised as follows :—

The intrinsic properties of the blood.
The renal factor.
Metabolic functions.
Respiration.

The Intrinsic Properties of the Blood.—That the blood, by nature of its physical and chemical properties, tends to keep its reaction constant, has been known for a very long time. Thus the addition of acids or alkalis, except in very large quantities, to blood does not alter its reaction. This property is known as buffering, and is due to the presence of buffer substances. In blood the most important buffers are the proteins and salts, such as the phosphates Na_2HPO_4 and NaH_2PO_4, all of which are capable of adding on acids or alkalis with the formation of compounds which do not give free H or OH ions in solution. The free H ions are soaked up, so to speak, and are consequently unable to influence the reaction. Sodium bicarbonate acts similarly in the blood, a

neutral salt being formed by the action of an acid, with the liberation of the weak acid H_2CO_3. This is readily excreted in the form of H_2O and CO_2.

We have, therefore, three substances acting as a buffer between the blood and excess hydrogen ions. These buffer substances constitute the true alkali reserve. It is usual to estimate alkali reserve in the form of sodium bicarbonate, since it is rightly assumed that any persistent increase in the hydrogen ions would attack this first, and a measure of the blood bicarbonate would indicate the extent of the acid condition, a low figure pointing to a serious state.

A depletion of the alkali reserve, such as described above, is termed an *acidosis*, whilst an increase is known as an *alkalosis*. These terms refer to the quantity of available alkali present in the blood, and do not refer to the hydrogen ion concentration of the blood. It must be clearly understood that a patient may be in an extreme condition of acidosis with a normal hydrogen ion concentration of the blood. Depletion in the alkali reserve precedes a rise in the hydrogen ion concentration, and, hence, is a valuable indication for treatment.

The methods for the estimation of alkali reserve are described at the end of this section.

The Renal Factor.—The remarkable power of the kidney to secrete urine of widely varying reaction from the blood of constant hydrogen ion concentration has always attracted attention. Bence-Jones (18) was one of the first to recognise the significance of this phenomenon, when he described the alkaline urinary tide following a meal. This he ascribed to the removal of HCl from the blood during gastric secretion, thus tending to leave the blood in a more alkaline condition.

The kidney then excretes alkaline urine in order to compensate the blood. Bence-Jones' theories have been borne out by a number of investigators, and the importance of the work has been fully recognised. The following can be taken as an example of the renal function in this respect.

According to Cushny's theories of renal secretion, certain

substances are kept at a more or less constant, or liminal, concentration in the blood. Amongst these we have certain salts, such as the two phosphates referred to above. Therefore, one of the renal functions would be to keep the

ratio $\frac{NaH_2PO_4}{Na_2HPO_4}$ constant.

Now any condition of acidosis will increase the value of this ratio, and, conversely, a condition of alkalosis will decrease its value. The kidney will respond by excreting either one or the other, thus restoring the value of the ratio to its original figure.

In this manner the kidney will excrete the absorbed hydrogen ions.

Metabolic Functions.—In general, the metabolic processes incline towards the production of acid. Thus sulphur is oxidised to sulphuric acid, and lactic and butyric acids are produced, but in one particular process alkali is formed, and is used to neutralise the acids. It will be remembered that proteins, after being digested, are absorbed into the portal blood stream in the form of amino-acids. On reaching the liver these acids are deaminated, resulting in the setting free of an oxyacid and ammonia. Under normal circumstances the greater part of the ammonia combines with CO_2 and water, carbonic acid being the strongest acid present, to form ammonium carbonate, which in turn is converted into urea by the removal of two molecules of water.

If, however, any stronger acid than H_2CO_3 be present, the ammonia combines with it to form an ammonium salt, thus constituting another factor in the neutrality regulating mechanism. A condition of acidosis will therefore raise the ammonia and decrease the urea content of the urine. This can best be expressed by working out the so-called ammonia co-efficient, *i.e.*, $\frac{\text{ammonia nitrogen}}{\text{total nitrogen}} \times 100$. Normally this is about 5%, and may be increased up to 10 to 18% in

extreme conditions of acidosis. A rise in the ammonia coefficient can be demonstrated very easily after starvation, either natural or due to vomiting.

Respiration.—One of the methods of absorbing acid is by means of sodium bicarbonate. As has been stated already, the action of acid is to produce a neutral salt and CO_2, or rather H_2CO_3.

Carbon dioxide stimulates the respiratory centre, with the result that the respiratory movements become much deeper, and consequently, the ventilation is more efficient, with the result that CO_2 is washed out of the blood. Any tendency to changes in the H ion concentration of the blood acts as a respiratory stimulus, producing exactly the same effects as CO_2. It appears that the body uses this weak volatile acid for fine adjustment of the blood reaction—a condition of acidosis being accompanied by washing out of acid in the form of CO_2, whilst an alkalosis is compensated for by retention of this substance.

Support for this view is obtained by studying the respiratory changes following a meal. If no food be taken for some time, the alveolar carbon dioxide tension was shown by Haldane (19) to remain more or less constant for the individual. If, however, a meal be taken, it was shown by one of us (E. C. D.) (20) that the CO_2 tension rises during gastric secretion, and falls during pancreatic secretion. The rise during gastric secretion denotes retention of CO_2, due to the alkalosis following the pouring out of HCl, whilst the fall corresponds to excretion of CO_2 in the period of acidosis following alkaline pancreatic secretion. A fall in the alveolar CO_2 tension indicates that the ventilation is increased, with the result that CO_2 is being washed out of the blood. This means a state of acidosis, whilst an increase indicates alkalosis. Normally the alveolar CO_2 tension varies from 35 to 45 mm., or, as a percentage, from 4 to 6·2. In acidosis the figures are much lower, 20 mm., or 1 to 2%.

When carbohydrate metabolism is deranged, as in diabetes mellitus, the fat metabolism is also affected.

Normally, the complex long chain fatty acids are broken down two carbon atoms at a time, until some simple substance, such as pyruvic acid, results, which is then oxidised to CO_2 and water. In diabetes, however, the breaking down process stops at the 4-carbon atom stage, thus leaving butyric acid. The body then tries to oxidise it away in the following series of reactions :

$CH_3CH_2CH_2COOH$	Butyric acid.
$CH_3CHOHCH_2COOH$	β-oxy-butyric acid.
CH_3COCH_2COOH	Aceto-acetic acid.
$CH_3COCH_3 + CO_2$	Acetone.

The presence of these bodies in sufficient concentration produces the clinical condition known as diabetic coma.

β-oxy-butyric and aceto-acetic acids are substances which ionise, and consequently are capable of producing acidosis. There is evidence of acidosis in coma, since the alveolar CO_2 tension and the alkali reserve are lowered, and the question arises as to whether the condition is due entirely to acidosis, or whether, as some observers think, there is a specific pharmacological action of acetone bodies.

Some authorities hold the former view, and prefer to call the condition in diabetes *acidosis*, whilst others lean to the specific action theory and refer to the condition as *ketosis*. It has been stated that coma can be produced by the administration of neutral salts of aceto-acetic acid. This effect is said to be due to the presence of an enolic group $\begin{pmatrix}COH\\CH.\end{pmatrix}$ (Hurtley and Trevan) (21). Against this is the fact that workers have imbibed large quantities of acetone bodies without any effect, whilst all observers estimate the alkali reserve with a view to ascertaining the onset of coma.

Methods of Investigating Acidosis and Ketosis

The practical importance of qualitative urinary tests for acetone bodies has already been emphasised, and it is only with the more elaborate procedures that we are concerned here. The following tests are usually performed on the patient in whom acidosis is suspected.

1. Estimation of alkali reserve by Van Slyke's method.
2. Determination of the alveolar carbon dioxide tension.

Van Slyke's Method for the Estimation of Alkali Reserve (22)

In this method the alkali reserve is estimated in the form of sodium bicarbonate. It consists in adding acid to a measured quantity of serum or plasma, and extracting the

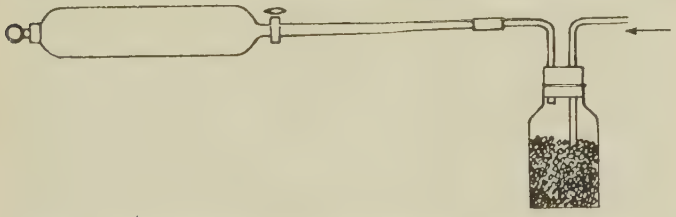


FIG. 1.—Apparatus for Saturating Plasma with CO_2 (Van Slyke). The bottle contains glass beads.

CO_2 so produced by reducing the pressure. The fluid is trapped by a very ingenious device, and the CO_2 is measured. The alkali reserve is calculated from this volume by reference to a table.

The actual CO_2 content of the blood, or else its CO_2 combining power, can be determined.

Solutions.—Phenolphthalein in alcohol (1%).

Caprylic alcohol.

5% sulphuric acid.

1% ammonia (carbonate free).

Blood is removed by vein puncture and is collected straight

into a centrifuge tube. The corpuscles are then centrifuged down, and as much plasma as possible is pipetted off into a separating funnel. This is placed on its side, and is filled with the operator's alveolar air. It is advisable to pass the breath through some form of condensation bottle first (see Fig. 1). The funnel is then rotated around its long axis in such a manner that the plasma is spread over the sides in the form of a thin film. Two minutes of this treatment are sufficient to saturate the plasma with CO_2. Whilst this is proceeding, the analysis apparatus is prepared. A line drawing of this appears on this page (Fig. 2), which is sufficiently clear without further explanation. The efficiency of the taps is tested by turning them off, and lowering the mercury reservoir, until a Torricellian vacuum is produced. If the taps are efficient, a sharp click will be heard on raising the reservoir. The taps F and E are set so that the reservoir B and trap D communicate with the main chamber A. The mercury reservoir is raised to position I when the mercury will just fill the capillary stem of B. The cup B is washed out with the ammonia solution, containing a drop of phenolphthalein solution. The washing is continued until the solution remains red, when all is

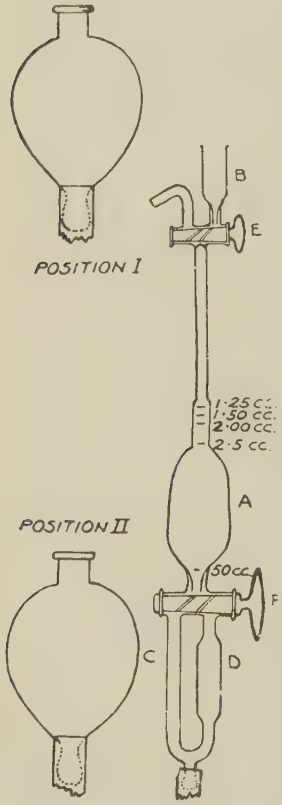


Fig. 2.—Van Slyke's Apparatus for the Estimation of Alkali Reserve.

The reservoir is connected to the main apparatus by means of a rubber tube, the ends of which are shown cut across.

pipetted off except a few drops. One c.c. of plasma is then run into the cup from an Ostwald pipette, the point of which is kept under the surface of the ammonia solution. The tap E is then closed, and the mercury reservoir is lowered to position II. The tap E is then cautiously opened, and the liquid contained in the cup is run into the main reservoir until the upper surface of the liquid reaches the top of the capillary stem, when the tap is shut. The cup is then washed with two lots of 0·5 c.c. distilled water, each washing being run into the main chamber as before. A small drop of caprylic alcohol is then sucked into the chamber, followed by 0·5 c.c. of the sulphuric acid solution. The tap E is then closed. The greatest care must be taken to avoid the introduction of air into the apparatus. The reservoir is now lowered still further, thus producing a Torricellian vacuum in the apparatus and causing CO_2 to be liberated. The mercury level inside the apparatus will now have fallen to the 50 c.c. mark, and the tap F is closed. The apparatus is removed from the stand, and is shaken, in order to ensure thorough liberation of gas. After replacing in the stand, the tap F is opened, and the solution is allowed to run into the trap D. When the upper surface of the liquid reaches the top of the capillary in the tap, it is rapidly reversed and the mercury reservoir raised. The mercury will then run into the main chamber *viâ* the tube C, leaving the liquid trapped in D. The height of the reservoir is so arranged that the levels inside and outside the apparatus are the same, when the volume of the gas is read off.

Despite the ingenious method of trapping, a minute quantity of water will collect above the mercury, but if the reading be made at once no correction need be applied for the amount of CO_2 absorbed by this minute volume. The volume of gas is then reduced to standard pressure by multiplying by the factor obtained from the Table I. given below, taking the figure corresponding to the barometer reading for the day. By means of Table II. the temperature factor is

Table I.

Barometer.	Barometer. 760	Barometer.	Barometer. 760
732	0·961	756	0·995
734	0·966	758	0·997
736	0·968	760	1·000
738	0·971	762	1·003
740	0·974	764	1·006
742	0·976	766	1·008
744	0·979	768	1·011
746	0·981	770	1·013
748	0·984	772	1·016
750	0·987	774	1·018
752	0·989	776	1·021
754	0·992	778	1·024

(Van Slyke.)

allowed for, and by means of the right hand columns the bicarbonate equivalent can be determined.

If a large number of determinations have to be made daily, it is advisable to install special apparatus. The saturation of plasma with CO_2 is effected by passing a mixture of 5·5% CO_2 in air from a cylinder (supplied by the British Oxygen Co.) through the plasma for five minutes after the addition of caprylic alcohol. If a long glass tube, with a number of side tubes at right angles, be employed, a number of sera can be saturated at once. This method standardises the saturation, and eliminates the individual factor of the alveolar air, besides saving the operator much fatigue. The Van Slyke apparatus should also be incorporated on a vertical board, hinged to the base-board of the apparatus. This is connected to a wheel by an eccentrically placed connecting rod, so that on rotation of the wheel by a motor, mechanical shaking of the apparatus occurs. This should be done

Table II.

Table for Calculation of CO_2—Combining Power for Plasma.

Observed Vol. Gas × $\frac{B}{760}$	c.c. of CO_2 reduced to O° 760 mm., bound as Bicarbonate by 100 c.c. of Plasma.				Observed Vol. Gas × $\frac{B}{760}$	c.c of CO_2 reduced to O° 760 mm., bound as Bicarbonate by 100 c.c. of Plasma.			
	15	20	25	30		15	20	25	30
0·20	9·1	9·9	10·7	11·8	0·60	47·7	48·1	48·5	48·6
1	10·1	10·9	11·7	12·6	1	48·7	49·0	49·4	49·5
2	11·0	11·8	12·6	13·5	2	49·7	50·0	50·4	50·4
3	12·0	12·8	13·6	14·3	3	50·7	51·0	51·3	51·4
4	13·0	13·7	14·5	15·2	4	51·6	51·9	52·2	52·3
5	13·9	14·7	15·5	16·1	5	52·6	52·8	53·2	53·2
6	14·9	15·7	16·4	17·0	6	53·6	53·8	54·1	54·1
7	15·9	16·6	17·4	18·0	7	54·5	54·8	55·1	55·1
8	16·8	17·6	18·3	18·9	8	55·5	55·7	56·0	56·0
9	17·8	18·5	19·2	19·8	9	56·5	56·7	57·0	56·9
0·30	18·8	19·5	20·2	20·8	0·70	57·4	57·6	57·9	57·9
1	19·7	20·4	21·1	21·7	1	58·4	58·6	58·9	58·8
2	20·7	21·4	22·1	22·6	2	59·4	59·5	59·8	59·7
3	21·7	22·3	23·0	23·5	3	60·3	60·5	60·7	60·6
4	22·6	23·3	24·0	24·5	4	61·3	61·4	61·7	61·6
5	23·6	24·2	24·9	25·4	5	62·3	62·4	62·6	62·5
6	24·6	25·2	25·8	26·3	6	63·2	63·3	63·6	63·4
7	25·5	26·2	26·8	27·3	7	64·2	64·3	64·5	64·3
8	26·5	27·1	27·7	28·2	8	65·2	65·3	65·5	65·3
9	27·5	28·1	28·7	29·1	9	66·1	66·2	66·4	66·2
0·40	28·4	29·0	29·6	30·0	0·80	67·1	67·2	67·3	67·1
1	29·4	30·0	30·5	31·0	1	68·1	68·1	68·3	68·0
2	30·3	30·9	31·5	31·9	2	69·0	69·1	69·2	69·0
3	31·3	31·9	32·4	32·8	3	70·0	70·0	70·2	69·9
4	32·3	32·8	33·4	33·8	4	71·0	71·0	71·1	70·8
5	33·2	33·8	34·3	34·7	5	71·9	72·0	72·1	71·8
6	34·2	34·7	35·3	35·6	6	72·9	72·9	73·0	72·7
7	35·2	35·7	36·2	36·5	7	73·9	73·9	74·0	73·6
8	36·1	36·6	37·2	37·4	8	74·8	74·8	74·9	74·5
9	37·1	37·6	38·1	38·4	9	75·8	75·8	75·8	75·4
0·50	38·1	38·5	39·0	39·3	0·90	76·8	76·7	76·8	76·4
1	39·1	39·5	40·0	40·3	1	77·8	77·7	77·7	77·3
2	40·0	40·4	40·9	41·2	2	78·7	78·6	78·7	78·2
3	41·0	41·4	41·9	42·1	3	79·7	79·6	79·6	79·2
4	42·0	42·4	42·8	43·0	4	80·7	80·5	80·6	80·1
5	42·9	43·3	43·8	43·9	5	81·6	81·5	81·5	81·0
6	43·9	44·3	44·7	44·9	6	82·6	82·5	82·4	82·0
7	44·9	45·3	45·7	45·8	7	83·6	83·4	83·4	82·9
8	45·8	46·2	46·6	46·7	8	84·5	84·4	84·3	83·8
9	46·8	47·1	47·5	47·6	9	85·5	85·3	85·2	84·8
0·60	47·7	48·1	48·5	48·6	1·00	86·5	86·2	86·2	85·7

(Van Slyke.)

for one minute. By this means every part of the estimation is standardised and consistent results are obtained.

The normal limits of CO_2 capacity were found by Van Slyke to be from 53 to 77 c.c. per 100 c.c. of plasma.

The following table gives a rough idea of the findings in various types of cases :—

	CO_2 capacity, c.c. per 100 c.c.
Thirty normal students . . .	52 to 79
Acidosis, mild	44
Acidosis, severe, in terminal stages of chronic interstitial nephritis . .	34
Acidosis, severe, after anæsthesia . .	32
Fatal diabetic coma	21
Diabetes without coma . . .	34

Determination of the Alveolar Carbon Dioxide Tension

The observer has a choice of several methods for this determination, but here we shall describe only three.

Collection of sample by the method of Haldane and Priestley, with subsequent analysis on Haldane's apparatus.

Fridericia's method.

The automatic method, using a katharometer.

Haldane's Method (23) is undoubtedly the ideal from the point of view of accuracy, but it requires considerable skill and experience. The collection of the samples for analysis is effected by a long rubber tube, about 6 feet long and 1 inch in diameter. This is fitted with a mouthpiece and side tube at one end. The side tube is connected either directly to the burette of the gas analysis apparatus, or else to an evacuated receiver.

The patient is instructed to breathe normally. At the end of an inspiration the operator tells him to breathe out as hard as he can down the tube, and, on reaching the limit of

this forced expiration, to close the end of the tube with his tongue. The operator then collects a sample through the side tube. This is analysed for CO_2, and the resulting percentage gives the CO_2 content of an inspiratory sample of alveolar air. The process is repeated, only the patient is told to blow down the tube at the end of a normal expiration. The CO_2 content of this expiratory sample will be higher than that of the former, and the mean of the two gives the required figure. This can be expressed in terms of tension by the following calculation :—

$$\left.\begin{matrix}CO_2 \text{ tension} \\ \text{in mm.}\end{matrix}\right\} = \frac{\text{percentage observed}}{100} \times \begin{matrix}\text{barometric pressure} \\ \text{in mm.}\end{matrix}$$

The method of analysis is described in Chapter VII. Although this method is almost perfect, provided that the operator is sufficiently skilful and the patient sufficiently sensible, it obviously has grave limitations. Thus it cannot be used for comatose or for dyspnœic patients. Again, some patients seem unable to understand the directions, and whatever one does a bad result is obtained. In the course of a long series of respiratory observations on patients, one of us (E. C. D.) employed a very simple and, at the same time, satisfactory method for the collection of samples of alveolar air. The patient was instructed to whistle rapidly through the lips, and when the note began to fall off the nozzle of a 20 c.c. syringe was pushed into the aperture between the lips, and a sample collected. This was immediately injected into the burette of the gas analysis apparatus. Although this method appears to break all the laws of respiratory technique, yet, on comparison with the standard Haldane-Priestley method, it was found to yield very accurate results. One hundred analyses were performed, and the two methods were compared. The figures were almost identical, except that the syringe method tended to give rather higher readings than the Haldane-Priestley method. The syringe method does away with masks, tubes, etc., and possesses the advantage that one can see what is

going on all the time. It must be realised, however, that the collection of samples of alveolar air is a very difficult matter, whatever method be adopted, and that it requires constant practice to get reliable samples.

Poulton (24) employs a very ingenious instrument, which he calls a tensimeter, for the collection of alveolar air from dyspnœic patients. With definitely comatose patients, the syringe method yields fairly good results, the breath being collected at the end of an expiration.

Fridericia's Method (25).—Here the air is collected and analysed in the apparatus shown in the illustration (see Fig. 3). The volume from A to B is 100 c.c. and from B to the bulb the tube is graduated in cubic centimetres (from 2 to 8), thus corresponding to percentages of the total volume. The apparatus requires no explanation other than the tap B, which is of such a nature that it can connect the chamber D with E, or D with the tube C, or E with C. Before a determination, the tap B is set so that D and E are connected, and the tap A is open ; thus the apparatus forms a U-tube.

The patient blows for as long as he possibly can through A, and at the end of the expiration A is shut. The whole apparatus is immersed in a pail of water in an upright position. After five minutes it is assumed that the air will have been cooled to the water temperature, and that no diffusion will have taken place. The apparatus is then removed from the pail, and the tap B is turned so that C and E communicate. By means of a rubber bulb attached to the free end of FE, 2 to 3 c.c. of 20% NaOH are sucked up into the tube E. The tap B is then turned sharply so that E and D communicate. By squeezing the bulb, alkali is forced into D, and the tap B is turned so as to shut off the main chamber D completely. The apparatus is then violently shaken for a minute, in which time it is assumed that all the CO_2 will have been absorbed. The apparatus is then replaced in the pail of water, and the tap B is turned under water so that D and C communicate.

After leaving for five minutes or so, it is found that water has entered the main chamber. The whole apparatus is then adjusted so that the levels inside and outside the chamber D

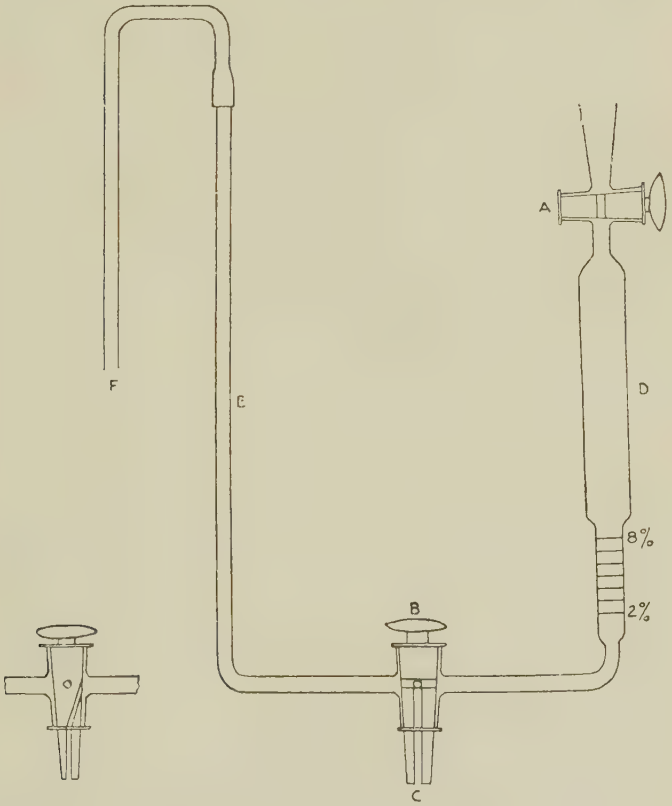


Fig. 3.—Fridericia's Apparatus for the Estimation of the Alveolar CO_2 Percentage.

are the same. The percentage of CO_2 can then be read off directly on the scale. Many observers state that this method is reliable and easy to perform, but our experience has been rather the reverse.

The Automatic Method.—The Cambridge Scientific Instrument Company has provided a new apparatus with which, it was stated, the percentage of CO_2 in the alveolar air could be read off automatically. The makers kindly supplied us with one of these instruments, and we were able to satisfy ourselves that it was sufficiently accurate for clinical purposes. The method is absolutely automatic and requires no skill whatsoever.

In conclusion, it must be thoroughly understood that the collection and analysis of alveolar air require great practice, and that a great many determinations must be made before reliable results are obtained. These remarks do not apply to the automatic method. The normal alveolar CO_2 tension lies somewhere between 35 and 45 mm. Below 30 mm. acidosis is suspected, while below 20 mm. the onset of coma should be expected.

In actual coma the figures may fall as low as 10 to 20 mm.

The Treatment of Diabetes Mellitus

When a copper-reducing substance is found in the urine of a patient, certain investigations must be carried out before the case can be pronounced one of diabetes. First the nature of the reducing substance must be determined. If this be found to be glucose, the sugar tolerance of the individual must be investigated in order to decide whether the case is one of diabetes mellitus. The methods employed in these proceedings have been described above.

These preliminary examinations apply only to mild cases of diabetes ; in severe types, with symptoms such as polyuria, thirst, wasting, and a marked degree of ketosis, the determination of sugar tolerance is hardly necessary from the point of view of diagnosis.

The starvation treatment of diabetes undoubtedly constituted a great therapeutic advance. It was introduced by Allen (26) in 1913, as the result of experiments on

partially depancreatised dogs, and was followed by a considerable improvement in the prognosis of the disease. Guelpa (27), in 1910, had advocated periodical days of starvation combined with purgation in the treatment of diabetes. Graham (28), in 1917, also evolved a method of treatment by starvation somewhat similar to that of Allen.

The isolation of insulin by Banting and Best (1) in 1922 constitutes a landmark, although it must be recognised that it is in no sense a cure, and in every instance its use must be combined with considerable dietetic restriction.

Since the introduction of insulin, there is no unanimity as to the best procedure in treating a case of diabetes. There are four chief methods at present in vogue.

1. The patient is starved until sugar free, if possible, and then the diet is built up with protein and fat together with a small amount of carbohydrate. Insulin is given in small doses, if required, in order to keep the blood sugar within normal limits.

2. In certain cases, good results may be obtained by the administration of insulin during the period of starvation. By this means it is possible to render the urine sugar free in a short time, and severe cases respond very well to this mode of treatment. It is essential, however, to keep a very careful watch on the patient, and frequent blood sugar determinations are necessary in order to avoid prolonged and dangerous hypoglycæmia.

3. The patient is not starved, but is put at once on what is known as the " basal requirement diet," and insulin is given in gradually increasing doses until the blood sugar falls within normal limits. In this method large doses of insulin, up to 60 units daily, may be necessary. Insulin should also be used at once in cases of diabetes with complications such as coma, tuberculosis, gangrene and neuritis.

4. Here no attempt is made to keep the blood sugar continuously within normal bounds, but fairly large quantities

of carbohydrate are given, together with insulin, so that there results only a slight glycosuria.

The method which from practical experience we have found to give the best results in moderate cases is the first of these. It possesses the following advantages :—

1. Insulin is not used unless necessary.
2. The pancreas is rested as much as possible.
3. The blood sugar is kept within normal limits.

In more severe cases, the second method should be employed. This is described later.

The Method.—The patient is put to bed and given an ordinary standard diet of known caloric value. The blood and urine sugars are estimated and the presence of ketosis noted. In this way the severity of one case can be compared with that of another, and the condition of the patient can be accurately contrasted at some time later, if required, with his state when treatment was first undertaken. The sugar tolerance is then determined as described above. If the condition of the patient be good, and there be no call for the immediate institution of measures directed to the control of the diabetes, a search should be made for any possible focus of sepsis. Attention should be directed to the mouth, the teeth should be X-rayed, and the tonsils, naso-pharynx and sinuses investigated by a throat specialist. Constipation should be corrected, the abdomen examined with a view to detecting infection of the gall-bladder or appendix, and the lungs for signs of tuberculosis. It has been shown that sepsis is often associated with hyperglycæmia, and possibly it is causally related thereto. The removal of septic foci is a matter of some importance in the treatment of diabetes. If an anæsthetic be required for this, as will probably be the case, it must be postponed until the patient is sugar free, as the result of the anti-diabetic measures proper. In acute cases, in which an immediate operation with an anæsthetic is imperative, special means of treatment should be adopted, which are detailed below.

When these investigations have been completed, an attempt must be made to render the patient sugar free, and to reduce the blood sugar to within normal limits by means of starvation. In severe cases in which there is marked acidosis, our experience has shown that the sudden deprivation of carbohydrate food may precipitate coma. In such instances the diet must be reduced gradually, the fats first being cut down, then the proteins, and finally the carbohydrates, or the patient may be starved and given insulin as described later. In the vast majority of cases, however, starvation can be undertaken with impunity, as the acidosis due to starvation is not toxic. It is usually noted that the acidosis increases in mild cases during the initial stages of starvation and the lower grade diets. During this period the patient is allowed as much fluid as is desired in the form of water, lemonade, weak tea or coffee (without sugar or milk), and half a pint of Bovril or Lemco is given twice daily. Small quantities of alcohol, in the form of whisky or brandy, may be given during this stage if desired. The urine usually becomes sugar free in twenty-four to forty-eight hours, but in some instances starvation has been carried out for as long as seven days. If it be not then sugar free, diet 1 should be commenced. Should any signs of coma appear they must be promptly treated with insulin and glucose, as detailed below, when a ready response will usually be obtained. When the patient is sugar free, or at the end of the maximum period of starvation deemed advisable, whichever shall first occur, the graduated diets are commenced. These are best prescribed in accordance with the individual requirements, and may be built up somewhat as follows :—

Graduated Diets in Diabetes

In building up a graduated diet for a diabetic patient who has been rendered sugar free, it is essential to know the constitution of the articles of food used as regards carbohydrate,

protein and fat. This is usually expressed as grams per ounce of foodstuffs.

The caloric value of each article in the diet can then be easily calculated, as 1 gram of carbohydrate or of protein is equivalent approximately to 4 Cals. and 1 gram of fat to 9 Cals.

The constitution of the more common articles of food, according to MacLean, is given in the following table :—

	Carbo-hydrate.		*Carbo-hydrate.*
Vegetables A. = 5%	..	1 oz. containing	1 g.
Vegetables B. = 10%	..	1 oz. ,,	2 g.
Vegetables C. = 15%	..	1 oz. ,,	3 g.
Vegetables D. = 20%	..	1 oz. ,,	4 g.
Vegetables E. = 30%	..	1 oz. ,,	6 g.

Vegetables A. include cabbage, cauliflower, Brussels sprouts, lettuce, spinach, cucumber, asparagus, marrow, rhubarb, celery, mushrooms, tomatoes, watercress, seakale, leeks, radishes, and grape fruit.

Vegetables B. include carrots, onions, turnips, beets, and French beans.

Vegetables C. include oranges, strawberries, gooseberries, cranberries, peaches, pineapple, and melon.

Vegetables D. include peas, artichokes, parsnips, pears, apples, currants, cherries, raspberries and apricots.

Vegetables E. include potatoes, plums, prunes and bananas.

		Grams.					
		Carbo-hydrate.		*Pro-tein.*		*Fat.*	*Calo-ries.*
Veg. A. (raw)	1 oz. =	1	..	0·5	..	0	= 6
Veg. B. ,,	1 oz. =	2	..	0·5	..	0	= 10
Veg. C. ,,	1 oz. =	3	..	2	..	0·1	= 21
Veg. D. ,,	1 oz. =	4	..	2	..	0·1	= 25
Veg. E. ,,	1 oz. =	6	..	1	..	0	= 28

		Grams.			
		Carbo-hydrate.	*Pro-tein.*	*Fat.*	*Calo-ries.*
Bread (white)	1 oz.	= 14·3	2	0·4	= 69
Bread (brown)	1 oz.	= 13	1·4	0·4	= 61
Oatmeal	1 oz.	= 18	4·6	2	= 108
Meat (uncooked lean)	1 oz.	= 0	6	3	= 51
Bacon	1 oz.	= 0	5	15	= 155
Fish (cod, whiting, haddock)	1 oz.	= 0	5	0	= 20
One egg	2 oz.	= 0	6	6	= 78
Butter	1 oz.	= 0	0	25	= 225
Cheese	1 oz.	= 0·1	8	10	= 122
Milk	1 oz.	= 1·5	1	1	= 19
Cream (20%)	1 oz.	= 1	1	6	= 62

The graduated diets may then be built up somewhat as follows :—

	Grams.			
Diet 1.	*Carbo-hydrate.*	*Pro-tein.*	*Fat.*	*Calo-ries.*
Veg. A., 10 oz.	10	5	0	60
Eggs, 2	0	12	12	156
Lemco, Bovril, tea, coffee (without sugar or milk), water as desired.				
Total =	10	17	12	216
Diet 2.				
Add Veg. A., 2 oz.	2	1	0	12
,, Eggs, 2	0	12	12	156
,, Butter, ½ oz.	0	0	12·5	112
Total =	12	30	36·5	496
Diet 3.				
Add Meat, 1 oz.	0	6	3	51
,, Energen bread, 2 rolls	4	3	0·5	32
Total =	16	39	40	579

	Grams.			
	Carbo-hydrate.	*Pro-tein.*	*Fat.*	*Calo-ries.*
Diet 4.				
Add Bacon, 1 oz. . .	0	5	15	155
,, Fish, 2 oz. . .	0	10	0	40
,, Butter, ½ oz. . .	0	0	12·5	112
,, Cheese, ½ oz. . .	0	4	5	61
Total =	16	58	72·5	947
Diet 5.				
Add Meat, 2 oz. . .	0	12	6	102
Total =	16	70	78·5	1,049
Diet 6.				
Add Oatmeal, ½ oz. . .	9	2·3	1	54
,, Milk, 2 oz. . .	3	2	2	38
Total =	28	74·3	81·5	1,141
Diet 7.				
Add Cream, ½ oz. . .	0·5	0·5	3	31
,, Meat, 2 oz. . .	0	12	6	102
Total =	28·5	86·8	90·5	1,274
Diet 8.				
Add Milk, 2 oz. . .	3	2	2	38
,, Oatmeal, ½ oz. . .	9	2·3	1	54
Total =	40·5	91·1	93·5	1,266
Diet 9.				
Add Bacon, 1 oz. . .	0	5	15	155
,, Brown bread, ½ oz. .	6·5	0·7	0·2	30
Total =	47	96·8	108·7	1,551
Diet 10.				
Add Meat, 1 oz. . .	0	6	3	51
,, Cream, ½ oz. . .	0·5	0·5	3	31
Total =	47·5	103·3	114·7	1,633
Diet 11.				
Add Brown bread, ½ oz. .	6·5	0·7	0·2	30
,, Fish, 1 oz. . .	0	5	0	20
Total =	54	109	114·9	1,683
Diet 12.				
Add Brown bread, ½ oz. .	6·5	0·7	0·2	30
,, Meat, 1 oz. . .	0	6	3	51
Total =	60·5	115·7	118·1	1,764

	Grams.			
	Carbo-hydrate.	*Pro-tein.*	*Fat.*	*Calo-ries.*
Diet 13.				
Add Butter, ½ oz. . .	0	0	12·5	112
,, Milk, 1 oz. . .	1·5	1	1	19
,, Brown bread, ½ oz. .	6·5	0·7	0·2	30
Total =	68·5	117·4	131·8	1,925
Diet 14.				
Add Cream, ½ oz. . .	0·5	0·5	3	31
,, Butter, ½ oz. . .	0	0	12·5	112
,, Brown bread, ½ oz. .	6·5	0·7	0·2	30
Total =	75·5	118·6	147·5	2,098

The carbohydrate foods may, of course, be interchanged according to taste, attention being paid to their carbohydrate values. Thus 1 oz. of vegetable B. can be substituted for 2 oz. of vegetable A., and 1 oz. of vegetable C. for 3 oz. of vegetable A., etc. Various condiments and sauces may also be used in small quantities to render the dish more attractive : beef tea and chicken broth may be substituted for Lemco and Bovril. Saccharin up to 3 gr. daily may be used for sweetening purposes.

The tables given above are intended as a guide to the manner in which a diet can be built up, but no hard and fast rules can be laid down, each case being treated to a certain extent individually.

The food allowed in the day should be divided up so that the chief carbohydrate-containing meals occur in the morning and the evening, This is especially of importance if insulin is being administered morning and evening.

The diets which are described above must be divided up for practical purposes so as to constitute the meals for the day, the menus of which will now be set out.

Diet 1.—**Breakfast :** Veg. A., 3 oz. ; Egg, 1 ; Tea. **Lunch :** Veg. A., 2 oz. ; Bovril, 10 oz. ; Water. **Tea :** Veg. A., 2 oz. ;

Tea. **Dinner :** Veg. A., 3 oz. ; Egg, 1 ; Bovril, 10 oz. ; Water. **Total calories = 216.**

Diet 2.—**Breakfast :** Veg. A., 3 oz. ; Egg, 1 ; Butter, ¼ oz. ; Tea. **Lunch :** Veg. A., 3 oz. ; Egg, 1 ; Bovril, 10 oz. ; Water. **Tea :** Veg. A., 3 oz. ; Egg, 1 ; Tea. **Dinner :** Veg. A., 3 oz. ; Egg, 1 ; Bovril, 10 oz. ; Water ; Butter, ¼ oz. **Total calories = 496.**

Diet 3.—**Breakfast :** Veg. A., 3 oz. ; Egg, 1 ; Butter, ¼ oz. ; Energen bread, 1 roll ; Tea. **Lunch :** Veg. A., 3 oz. ; Egg, 1 ; Bovril, 10 oz. ; Water. **Tea :** Veg. A., 3 oz. ; Egg, 1 ; Tea. **Dinner :** Veg. A., 3 oz. ; Egg, 1 ; Bovril, 10 oz. ; Butter, ¼ oz. ; Meat, 1 oz. ; Energen bread, 1 roll ; Water. **Total calories = 579.**

Diet 4.—**Breakfast :** Veg. A., 3 oz. ; Egg, 1 ; Butter, ¼ oz. ; Energen bread, 1 roll ; Tea ; Bacon, 1 oz. **Lunch :** Veg. A., 3 oz. ; Egg, 1 ; Bovril, 10 oz. ; Water ; Butter, ¼ oz. ; Cheese, ½ oz. **Tea :** Veg. A., 3 oz. ; Egg, 1 ; Tea ; Butter, ¼ oz. **Dinner :** Veg. A., 3 oz. ; Egg, 1 ; Butter, ¼ oz. ; Meat, 1 oz. ; Energen bread, 1 roll ; Fish, 2 oz. ; Water. **Total calories = 947.**

Diet 5.—**Breakfast :** Veg. A., 3 oz. ; Egg, 1 ; Butter, ¼ oz. ; Energen bread, 1 roll ; Tea ; Bacon, 1 oz. **Lunch :** Veg. A., 3 oz. ; Egg, 1 ; Bovril, 10 oz. ; Water ; Butter, ¼ oz. ; Cheese, ½ oz. ; Meat, 3 oz. **Tea :** Veg. A., 3 oz. ; Egg, 1 ; Tea ; Butter, ¼ oz. **Dinner :** Veg. A., 3 oz. ; Egg, 1 ; Butter, ¼ oz. ; Energen bread, 1 roll ; Fish, 2 oz. ; Water. **Total calories = 1,049.**

Diet 6.—**Breakfast :** Veg. A., 3 oz. ; Egg, 1 ; Butter, ¼ oz. ; Energen bread, 1 roll ; Tea ; Bacon, 1 oz. ; Oatmeal, ½ oz. ; Milk, 2 oz. **Lunch :** Veg. A., 3 oz. ; Egg, 1 ; Bovril, 10 oz. ; Water ; Butter, ¼ oz. ; Cheese, ½ oz. ; Meat, 3 oz. **Tea :** Veg. A., 3 oz. ; Egg, 1 ; Tea ; Butter, ¼ oz. **Dinner :** Veg. A., 3 oz. ; Egg, 1 ; Butter, ¼ oz. ; Energen bread, 1 roll ; Fish, 2 oz. ; Water. **Total calories = 1,141.**

Diet 7.—**Breakfast :** Veg. A., 3 oz.; Egg, 1; Butter, ¼ oz.; Energen bread, 1 roll; Tea; Bacon, 1 oz.; Oatmeal, ½ oz.; Milk, 2 oz.; Cream, ½ oz. **Lunch :** Veg. A., 3 oz.; Egg, 1; Bovril, 10 oz.; Water; Butter, ¼ oz.; Cheese, ½ oz.; Meat, 4 oz. **Tea :** Veg. A., 3 oz.; Egg, 1; Tea; Butter, ¼ oz. **Dinner :** Veg. A., 3 oz.; Egg, 1; Butter, ¼ oz.; Energen bread, 1 roll; Fish, 2 oz.; Water; Meat, 1 oz.; Coffee. **Total calories = 1,274.**

Diet 8.—**Breakfast :** Veg. A., 3 oz.; Egg, 1; Butter, ¼ oz.; Energen bread, 1 roll; Tea; Bacon, 1 oz.; Oatmeal, 1 oz.; Milk, 3 oz.; Cream, ½ oz. **Lunch :** Veg. A., 3 oz.; Egg, 1; Bovril, 10 oz.; Water; Butter, ¼ oz.; Cheese, ½ oz.; Meat, 4 oz. **Tea :** Veg. A., 3 oz.; Egg, 1; Tea; Butter, ¼ oz. **Dinner :** Veg. A., 3 oz.; Egg, 1; Butter, ¼ oz.; Energen bread, 1 roll; Fish, 2 oz.; Water; Meat, 1 oz.; Milk, 1 oz.; Coffee. **Total calories = 1,366.**

Diet 9.—**Breakfast :** Veg. A., 3 oz.; Egg, 1; Butter, ¼ oz.; Energen bread, 1 roll; Tea; Bacon, 2 oz.; Oatmeal, 1 oz.; Milk, 3 oz.; Cream, ½ oz. **Lunch :** Veg. A., 3 oz.; Egg, 1; Bovril, 10 oz.; Water; Butter, ¼ oz.; Cheese, ½ oz.; Meat, 4 oz. **Tea :** Veg. A., 3 oz.; Egg, 1; Tea; Butter, ¼ oz.; Energen bread, 1 roll. **Dinner :** Veg. A., 3 oz.; Egg, 1; Butter, ¼ oz.; Fish, 2 oz.; Water; Meat, 1 oz.; Milk, 1 oz.; Brown bread, ½ oz.; Coffee. **Total calories = 1,551.**

Diet 10.—**Breakfast :** Veg. A., 3 oz.; Egg, 1; Butter, ¼ oz.; Energen bread, 1 roll; Tea; Bacon, 2 oz.; Oatmeal, 1 oz.; Milk, 3 oz.; Cream, ½ oz. **Lunch :** Veg. A., 3 oz.; Egg, 1; Bovril, 10 oz.; Water; Butter, ¼ oz.; Cheese, ½ oz.; Meat, 4 oz. **Tea :** Veg. A., 3 oz.; Egg, 1; Tea; Butter, ¼ oz.; Energen bread, 1 roll; Cream, ½ oz. **Dinner :** Veg. A., 3 oz.; Egg, 1; Butter, ¼ oz.; Fish, 2 oz.; Water; Meat, 2 oz.; Milk, 1 oz.; Brown bread, ½ oz.; Coffee. **Total calories = 1,633.**

Diet 11.—**Breakfast :** Veg. A., 3 oz. ; Egg, 1 ; Butter, ¼ oz. ; Tea ; Bacon, 2 oz. ; Oatmeal, 1 oz. ; Milk, 3 oz. ; Cream, ½ oz. ; Brown bread, ½ oz. **Lunch :** Veg. A., 3 oz. ; Egg, 1 ; Bovril, 10 oz. ; Water ; Butter, ¼ oz. ; Cheese, ½ oz. ; Meat, 4 oz. **Tea :** Veg. A., 3 oz. ; Egg, 1 ; Tea ; Butter, ¼ oz. ; Energen bread, 2 rolls ; Cream, ½ oz. **Dinner :** Veg. A., 3 oz. ; Egg, 1 ; Butter, ¼ oz. ; Fish, 3 oz. ; Water ; Meat, 2 oz. ; Milk, 1 oz. ; Brown bread, ½ oz. ; Coffee. **Total calories = 1,683.**

Diet 12.—**Breakfast :** Veg. A., 3 oz. ; Egg, 1 ; Butter, ¼ oz. ; Tea ; Bacon, 2 oz. ; Oatmeal, 1 oz. ; Milk, 3 oz. ; Cream, ½ oz. ; Brown bread, ½ oz. **Lunch :** Veg. A., 3 oz. ; Egg, 1 ; Bovril, 10 oz. ; Water ; Butter, ¼ oz. ; Cheese, ½ oz. ; Meat, 4 oz. **Tea :** Veg. A., 3 oz. ; Egg, 1 ; Tea ; Butter, ¼ oz. ; Energen bread, 2 rolls ; Cream, ½ oz. **Dinner :** Veg. A., 3 oz. ; Egg, 1 ; Butter, ¼ oz. ; Fish, 3 oz. ; Water ; Meat, 3 oz. ; Milk, 1 oz. ; Brown bread, 1 oz. ; Coffee. **Total calories = 1,764.**

Diet 13.—**Breakfast :** Veg. A., 3 oz. ; Egg, 1 ; Butter, ½ oz. ; Tea ; Bacon, 2 oz. ; Oatmeal, 1 oz. ; Milk, 3 oz. ; Cream, ½ oz. ; Brown bread, ½ oz. **Lunch :** Veg. A., 3 oz. ; Egg, 1 ; Bovril, 10 oz. ; Water ; Butter, ½ oz. ; Cheese, ½ oz. ; Meat, 4 oz. ; Brown bread, ½ oz. **Tea :** Veg. A., 3 oz. ; Egg, 1 ; Tea ; Butter, ¼ oz. ; Energen bread, 2 rolls ; Cream, ½ oz. ; Milk, 1 oz. **Dinner :** Veg. A., 3 oz. ; Egg, 1 ; Butter, ¼ oz. ; Fish, 3 oz. ; Water ; Meat, 3 oz. ; Milk, 1 oz. ; Brown bread, 1 oz. ; Coffee. **Total calories = 1,925.**

Diet 14.—**Breakfast :** Veg. A., 3 oz. ; Egg, 1 ; Butter, ½ oz. ; Tea ; Bacon, 2 oz. ; Oatmeal, 1 oz. ; Milk, 3 oz. ; Cream, 1 oz. ; Brown bread, 1 oz. **Lunch :** Veg. A., 3 oz. ; Egg, 1 ; Bovril, 10 oz. ; Water ; Butter, ½ oz. ; Cheese, ½ oz. ; Meat, 4 oz. ; Brown bread, ½ oz. **Tea :** Veg. A., 3 oz. ; Egg, 1 ; Tea ; Butter, ½ oz. ; Energen bread, 2 rolls ; Cream, ½ oz. ; Milk, 1 oz. **Dinner :** Veg. A., 3 oz. ; Egg, 1 ;

Butter, ½ oz.; Fish, 3 oz.; Water; Meat, 3 oz.; Milk, 1 oz.; Brown bread, 1 oz.; Coffee. **Total calories = 2,098.**

If feasible, blood sugar estimations should be made daily, either before breakfast or at some fixed hour after a meal. The urine sugar excretion should also be estimated daily and tests made for ketone bodies. These observations should be recorded on a chart.

According to the progress made by the patient, so is the dietary gradually increased, but it is well not to hurry the initial stages in order that the pancreas may be rested as much as possible. Whether or not insulin is required depends upon the capacity for taking an adequate dietary without it, the blood sugar being kept within normal limits and no sugar being found in the urine. The diets are increased until either the blood sugar rises or glycosuria appears, when the patient is starved for another day and again given the highest dietary which was satisfactory before. If it be impossible to increase this further, insulin must be used if the dietary is not yet adequate. Five units should first be given twice a day, half an hour before breakfast and supper. The clinical unit of insulin was originally described as one-third that amount of insulin which will lower the blood sugar of a rabbit weighing 2 kg., and starved for twenty-four hours, to 0·045% within four hours. The recent international unit is 40% stronger than this. This is usually sufficient to produce convulsions in a rabbit. The insulin and dietary are then increased until the patient is on a diet 20% in excess of his basal requirement, and is at the same time sugar free and with a normal blood sugar content. We have observed that whereas in some chronic cases of diabetes the renal threshold is above normal before treatment, it may fall below normal during treatment, with the result that there is glycosuria, although the blood sugar is within normal limits. This has also been noted by other observers such as Langdon Brown (29). When the diet and insulin dosage have been

thus worked out the blood sugar need not be estimated so frequently, but the urine should be tested daily.

It is necessary to know what constitutes an adequate dietary. The *basal requirement diet* varies with the weight and age of the patient. For an adult 12 to 15 calories for every pound of body weight are required, *i.e.*,

8 st.	. . .	1,680 Cals. at	15 Cals. per lb.
9 st.	. . .	1,890 Cals.	,, ,,
10 st.	. . .	2,100 Cals.	,, ,,
11 st.	. . .	2,310 Cals.	,, ,,
12 st.	. . .	2,520 Cals.	,, ,,

Women require 10% less than adult men, children require proportionately more. These figures are the minimum, and do not allow for anything more than the lightest muscular activity. In order that muscular work may be performed and weight maintained, additional calories will be required to the extent of 10 or 20%. The proportion of carbohydrate, fat and protein in the dietary is somewhat arbitrarily fixed as follows :—The carbohydrate tolerated by a diabetic, unless it be a very mild case, will not be more than 30 to 60 g. daily. One-half to $\frac{3}{4}$ g. of protein per pound of body weight is required. The remainder of the caloric value is made up by fat.

Woodyatt (30) says that the carbohydrate should bear a definite ratio to the fat on account of its supposed antiketogenous value ; this may be expressed by a formula

$$F < 2C + \frac{P}{2},$$ or a modified ratio of 1 carbohydrate to 1·3 fat

may be used as suggested by Banting (31). Excess of fat in the diet not only is a dangerous source of ketosis, but also appears to lead to hyperglycæmia, and so in diabetes, just as there is a disturbance in the metabolism of all three of the foodstuffs, so all three must be strictly limited in the prescribed diets.

In order to avoid the ketogenous properties of fats, Max

Kahn synthetised a fatty acid with an uneven number of carbon atoms which was incapable of giving rise to acetone bodies when administered. This substance, margaric acid, $C_{16}H_{33}COOH$, is combined with glycerine to form an ester, and the result, *intarvin*, can be given to diabetic patients without fear of producing acidosis. The substance is unpalatable, and although good results have been recorded, its use has been rendered unnecessary by the introduction of insulin.

A full account of the literature, and of the value of intarvin, can be found in a paper by Murray Lyon, Robson and White (32).

In 1926, Frank, Nothmann and Wagner (33) described a synthetic diguanidyl derivative of a long-chain aliphatic hydrocarbon, which they called *synthalin*, for oral administration in the treatment of diabetes. Careful clinical and laboratory experiments showed that the compound was toxic, and although the blood and urine sugar content could be reduced by its administration in mild cases, its action was not "insulin-like," and probably, therefore, not antidiabetic in the true sense. Its use has been abandoned in this country by most workers (34).

As regards the calculation of the basal requirement diet, the "predicted" body weight may be used instead of the actual body weight. This is of advantage, as thereby the very thin patient will be allowed a rather higher diet, and the obese patient one of a lower caloric value. It is also of use in those patients whose weight is unreliable on account of œdema. In this method the body length is taken with the patient sitting on the floor with knees bent and back against a wall. The height on the wall to the top of his head is measured.

Ainley Walker and Dreyer's (35) table gives the calculated body weight for each sex according to body length. Dreyer's (35) basal metabolism tables give the calories required for different weights at different ages, and these may be used instead of the factor of 15 calories per pound.

Basal Metabolism (*Dreyer*).

Calories—Males.

Weight.	Ages.									
	5	10	15	20	30	40	50	60	70	80
lb.										
10 . . .	536	—	—	—	—	—	—	—	—	—
20 . . .	758	390	657	—	—	—	—	—	—	—
30 . . .	928	846	804	773	733	704	683	667	653	642
45 . . .	1138	1037	986	946	898	863	838	818	801	788
60 . . .	1315	1198	1140	1094	1038	998	969	945	926	910
80 . . .	1517	1382	1315	1262	1197	1151	1117	1090	1068	1050
100 . . .	1693	1543	1467	1408	1336	1285	1247	1217	1192	1172
120 . . .	—	1689	1606	1542	1463	1407	1367	1332	1305	1283
140 . . .	—	1827	1738	1667	1582	1521	1476	1440	1411	1388
160 . . .	—	—	1858	1785	1692	1626	1578	1540	1509	1483
180 . . .	—	—	—	1889	1792	1723	1672	1632	1599	1571
200 . . .	—	—	—	2020	1917	1843	1789	1746	1710	1681

Relation Between Body Length and Body Weight (*Ainley Walker and Dreyer*).

Body Length (inches)	Body Weight.	
	Males.	Females.
20 . . .	24 . .	24
22 . . .	32 . .	32
24 . . .	42 . .	43
26 . . .	54 . .	55
28 . . .	68 . .	70
30 . . .	84 . .	88
32 . . .	103 . .	108
34 . . .	125 . .	131
36 . . .	150 . .	157
38 . . .	177 . .	186
40 . . .	208 . .	219

Basal metabolism is expressed as calories per twenty-four hours. In the case of females the values are 10% lower.

Simultaneous Starvation and Administration of Insulin

The procedure of administering insulin during starvation has already been referred to. This method is particularly suited to severe cases, but it is essential to have access to a laboratory where the blood sugar can be estimated at any time ; the method also requires considerable experience.

The patient is starved as soon as the preliminary tests already described have been performed. Insulin is given at intervals throughout the day. If the blood sugar be very high, say 400 to 500 mg. per 100 c.c., 50 to 60 units may be given in either two or three doses spaced evenly throughout the day. The urine should be collected two-hourly, and examined for sugar. The sugar usually disappears from the urine in twelve to forty-eight hours. As soon as the glycosuria disappears, the treatment is carried on as before, employing graduated diets. The insulin may frequently be omitted whilst the lower diets are being taken. The case is then treated in exactly the same manner as described under the orthodox method. Although very efficient, it can be seen that this method is liable to produce hypoglycæmia during the starvation period. Provided that the condition is recognised and treated, no harm will result, but whenever there is any doubt about the patient's condition, a blood sugar estimation should be performed.

The Use of Insulin without Blood Sugar Estimations

The necessity for frequent blood sugar estimations during the use of insulin in the treatment of diabetes would constitute a very serious drawback to its employment in general practice. Attempts have therefore been made to devise a method whereby the beneficial effects of insulin may be obtained without recourse to blood sugar determinations and without at the same time exposing the patient to the dangers of hypoglycæmia.

In any case, one preliminary blood sugar estimation

should be made in order to determine that hyperglycæmia is present, and so that the patient is suffering from diabetes and not from some other condition such as renal glycosuria. In very severe cases, however, in which acidosis is marked, a blood sugar reading is not essential, although advisable. In moderate degrees of diabetes the sugar tolerance curve should be worked out as described on p. 75, as a preliminary measure.

The following line of treatment may then be adopted : The patient is starved and worked up to his calculated " basal requirement diet " by means of the graduated diets described earlier in this chapter. If sugar be present in the urine when this stage is reached insulin injections should be begun. In more severe cases the simultaneous use of insulin with dietetic restrictions should be instituted at the start of the treatment, as described in a previous section of this chapter.

The diet should be so arranged that the greater part of the carbohydrate allowed each day is given in the morning and evening, whereas the two intermediate meals contain only very small quantities.

A dose of 5 units of insulin is then injected half an hour before breakfast and the urine subsequently passed is collected, as Graham (36) suggests, in three-hourly specimens throughout the day and in one night specimen of six hours' duration. Each of these specimens is tested for sugar, and the results may be recorded upon a chart. The *régime* would therefore be as follows : 9 a.m., collection of urine followed by injection of insulin ; 9.30 a.m., breakfast ; 12 noon, urine collected for previous three hours ; 1 p.m., lunch ; 3 p.m., urine collected for previous three hours ; 4.30 p.m., tea ; 6 p.m., urine collected for previous three hours ; 6.30 p.m., dinner ; 9 p.m., urine collected for previous three hours ; 12 midnight, urine collected for previous three hours ; 6 a.m., urine collected for previous six hours. If none of the three-hourly specimens of urine be sugar free after the 5 units of insulin the dose should be increased next morning to 10 units.

It may now be found that the second specimen after the injection (12—3 p.m.) is sugar free, although the first (9 a.m. —12) and third (3 p.m.—6 p.m.) specimens contain sugar, as do also the subsequent ones. In such a case the same dose of insulin should be persisted in for two or three days in order to see whether the sugar tolerance improves. If sugar be still passed during some part of the twenty-four hours a second injection of 5 units of insulin should be given at 6 p.m. after collecting the urine, and half an hour before dinner. The evening injection is made at least six hours before the patient goes to sleep, as the resulting fall in the blood sugar has usually passed off in this time, and so there is only a very remote danger of hypoglycæmia developing during sleep.

The doses of insulin can be gradually worked up, increasing by 5 units in the morning and next by 4 units in the evening alternately, until all the specimens of urine are sugar free. A smaller dose of insulin must always be given in the evening, as the carbohydrate content of the evening meal is less than that of the morning one. An interval of two or three days should elapse between each morning and evening increment in insulin dosage, and in this way the danger of giving an overdose is reduced to a minimum. In some instances it will be found that the three-hours' specimen, following the two chief carbohydrate-containing meals of the day, contains a little sugar, although the remainder are sugar free. In any case, it is not advisable to give a single dose of more than 30 units of insulin unless blood sugar estimations are being made, except as an emergency measure in diabetic coma. If glycosuria, therefore, persist when the insulin has been increased to 25 or 30 units in the morning and 17 to 19 units in the evening, the carbohydrate in the diet should be gradually reduced. A specimen of urine that is sugar free immediately before an injection of insulin is due is a warning that great caution should be used in increasing the dose. Increments of only 1 unit at a time are then

safest, and the patient should be warned of the early symptoms of hypoglycæmia (see p. 130) so that he may immediately counteract them by eating one or two lumps of sugar. Even when all the specimens of urine are sugar free, some authorities advise that the insulin should be still further cautiously increased, unit by unit, until early hypoglycæmic symptoms appear. If it be found that they occur a certain time after the injection of insulin, they can be averted by taking a biscuit about half an hour before they are expected, keeping at the same time the diet and the insulin dosage constant. If, however, they still persist, the insulin dosage should be gradually reduced until no hypoglycæmic symptoms are noticed (Leyton (37)).

Although it is possible to keep a patient almost, if not quite, sugar free by this method, yet it is not possible to know whether the maximum amount of rest is being afforded to the pancreas, as is the case when the blood sugar is maintained within its normal limits, as shown by repeated blood sugar estimations. This is so because, as we have seen above, the renal threshold may vary during the course of diabetes, so that in the early stages, if it be raised, the urine may be sugar free, although the blood sugar concentration is above the normal renal leak point of 180 mg. per 100 c.c.

If, on the other hand, during treatment the renal leak point fall below normal, the urine may contain sugar although the blood sugar is within normal limits. In such a case there is a possibility that hypoglycæmic symptoms might occur when just sufficient insulin is given to render the urine sugar free.

The most important factors in the successful administration of insulin without blood sugar estimations are the maintenance of a constant diet and the slow increase in the insulin injections until the correct dosage is established. Provided that the patient is aware of the early symptoms of hypoglycæmia, and has at hand the necessary antidote in the form of sugar. there is little to be feared on this score.

After-Treatment in Diabetes

The treatment which has just been described may be considered as the preliminary or stabilising treatment in diabetes: it consists in establishing an adequate dietary for the patient, so that the urine remains sugar free and the blood sugar is within normal limits.

As, however, the after-treatment is equally important, there are several points on which advice must be given before the patient leaves the shelter of constant medical supervision.

It is essential, first, that he shall understand the nature of the disease from which he is suffering and the fundamental principles of its treatment. These can be explained in simple terms intelligible to the layman. If he is taking insulin he should also be instructed in the method of its administration before he resumes his ordinary home life. Advice should be given on the following points :—

The Supply of Insulin.—This is sold in small rubber-capped bottles. The insulin should be stored in a cool, dark place: it will keep for several months, but should only be used if perfectly clear. Twenty units are contained in each c.c., but a double-strength insulin (40 units per c.c.) is available.

The Insulin Syringe.—A specially graduated syringe is simple to use. The graduations are in units, each division being $\frac{1}{20}$ c.c. The syringe may be kept in a metal case filled with proof spirit, which should be renewed each week to prevent the needle rusting. A stainless steel needle, size 16 to 18, may be employed. With such a syringe the necessity of sterilisation by boiling is abolished. As the rubber cap of the insulin bottle is apt to blunt the needle, it is preferable to employ two needles, one of which is used for piercing the rubber cap and filling the syringe, and the other for injecting the insulin. The former may be of larger gauge than the latter, and can be kept separately in a small glass-covered vessel containing spirit. The needles should be sharpened from

time to time on a piece of curved carborundum, as described in Chapter XV. The injections may be made into the arms or thighs, but the skin on the arms cannot be pinched up on inserting the needle if the patient give his own injection. If a local reaction, either urticarial or hæmorrhagic, result from the insulin injection, another brand should be tried, until one is found which does not cause it. The reaction is due to the protein in the insulin, as some brands are made from beef and others from pig pancreas.

The necessity for taking carbohydrate food within half an hour after a dose of insulin must be emphasised, but the diet and dosage of insulin will have been worked out before the patient begins his after-treatment.

Urine Examinations.—A specimen of urine should be examined on retiring at night and on rising in the morning. The patient should be taught to test the urine by Benedict's method. He should also test a specimen daily with ferric chloride for acetone bodies, and if these be present he should at once inform his doctor. Rothera's test is so delicate that minute quantities of acetone bodies may often be found, which are not of serious significance. If both specimens remain sugar free, the insulin dosage should be reduced unit by unit, or a little more carbohydrate added to the food until sugar just appears. The object is to keep the urine sugar free and at the same time reduce the insulin or increase the carbohydrate as much as possible in accordance with any improvement in the sugar tolerance.

A blood sugar estimation should be carried out once a month in the early stages of the after-treatment, and later at progressively longer intervals. This is the only possible way of knowing that the blood sugar is within normal limits, as the renal threshold for sugar excretion may change in the same patient during treatment, as previously described.

Symptoms of Hypoglycæmia.—The patient should be told clearly the significance of hypoglycæmia, and the symptoms by which he may recognise its onset. He should always

carry about two or three lumps of sugar which he can eat if he feels any such early symptoms as sweating or faintness after an injection of insulin. It is a debateable point whether or not every patient who is having insulin should receive a deliberate overdose during the initial stage of treatment, in order to familiarise himself with the symptoms of hypoglycæmia, and the ease with which they can be abolished in the early stages by appropriate treatment. It is not our custom to do so.

Exercise.—Patients who are taking insulin should not indulge in violent exercise, as hypoglycæmic coma has been known to occur in such cases.

Diet.—The patient at the completion of his initial or stabilising course of treatment is given a definite dietary sufficient in caloric value to maintain his energy requirements, and containing enough carbohydrate to enable his muscles, voluntary and involuntary, to carry out their respective functions. The remainder of his diet is made up of protein or fat in the correct proportion as detailed in a previous section of this chapter.

The patient must understand the cardinal principles of the composition of his diet, and learn to weigh out his food carefully. For this purpose a small pair of scales, weighing accurately to $\frac{1}{4}$ oz., is required. The household kitchen scales are not sufficiently delicate for the purpose. The fluid articles of diet may be measured in a graduated medicine glass. It is generally found that after a time the diabetic becomes a remarkably good judge of the weight of various articles of food, so that it is not essential at all times to have them weighed. Thus if he dine out or stay for a short time at a hotel, he can gauge very accurately the amount of meat, fish or vegetables he is allowed. This practice should not, however, be encouraged as a routine, as it favours slipshod habits which will sooner or later prove his undoing.

A very important consideration in the after-treatment of

a diabetic patient is the variation in diet which he is allowed.

Variations in Diet.—During the initial stages in treatment, when the individual dietary is being built up, patients must be, and usually are, content to take the graduated diets as laid down in the tables above, but when the amount of carbohydrate, protein and fat has been determined, which, with slight variations according to their health and advancing years, must be their lot for the remainder of their life, no effort should be spared in making the diet as varied and attractive as possible. A simple method of doing this is to consider each meal separately according to the carbohydrate, protein and fat content, and then from a table of diets to work out the most important equivalents.

Thus if the patient start his controlled diabetic life on diet 14, he may have his food apportioned as follows :—

	Grams.		
	C.	*P.*	*F.*
Breakfast	39·5	27·5	59·9
Lunch	9·5	36·2	35·7
Tea	9	12	23
Dinner	17·5	42·9	28·9
	75·5	118·6	147·5

The routine breakfast consists of Veg. A., 3 oz. ; Egg, 1 ; Butter, ½ oz. ; Tea ; Bacon, 2 oz. ; Oatmeal, 1 oz. ; Milk, 3 oz. ; Cream, 1 oz. ; Brown bread, 1 oz.

Lunch.—Veg. A., 3 oz. ; Egg, 1 ; Bovril, 10 oz. ; Water ; Butter, ½ oz. ; Cheese, ½ oz. ; Meat, 4 oz. ; Brown bread, ½ oz.

Tea.—Veg. A., 3 oz. ; Egg, 1 ; Tea ; Butter, ½ oz. ; Energen bread, 2 rolls ; Cream, ½ oz. ; Milk, 1 oz.

Dinner.—Veg. A., 3 oz. ; Egg, 1 ; Butter, ½ oz. ; Fish, 3 oz. ; Water ; Meat, 3 oz. ; Milk, 1 oz. ; Brown bread, 1 oz. ; Coffee.

The meals may be modified as follows :—

Breakfast.—The standard diet may be varied by the substitution of some of the following articles : an orange, an apple, ham, kippers, herrings, sardines, toast, smoked haddock and kidneys. Sausages are best avoided, as they contain a variable amount of bread. The composition of these articles of food is shown below :—

		Grams.			
		C.	*P.*	*F.*	*Calories.*
Orange (average weight with skin = 5 oz.)		12·5	1·2	0	55
Apple (average weight with skin = 4½ oz.)		15	0	0	60
Ham (boiled)	1 oz. =	0	6·6	6·6	86
Kippers (boiled)	1 oz. =	0	5	2	38
Herrings (cooked, fresh)	1 oz. =	0	7·5	3	57
Sardines (tinned)	1 oz. =	0	6·6	5·3	74
Smoked haddock (boiled)	1 oz. =	0	5	0	20
Kidneys (cooked)	1 oz. =	0	5	1·5	33·5
Toast	1 oz. =	20	2	0	88

Thus instead of porridge the 18 g. of carbohydrate allowed may be made up by an orange or an apple, together with the requisite amount of toast or veg. A., as determined by the above table. If the porridge be omitted there will remain 4·6 g. of protein and 2 g. of fat to be added. This can be effected by taking ⅔ oz. of bacon. If ham, kippers, herrings, sardines or smoked haddock be substituted for bacon, the fat lost can be made good by adding just under ½ oz. of butter. A small quantity of special marmalade, such as Callard's diabetic preparation, can also be eaten.

Lunch.—Variations in this meal may be made by using some of the following articles of food : brazil nuts, pickles, potato, special cheeses such as Gruyère, Gorgonzola, Stilton, and Camembert, chicken, fish instead of meat, stewed rabbit,

sweetbreads, tinned ox tongue, and olive oil. Their composition is as follows :—

		Grams.			
		C.	*P.*	*F.*	*Calories.*
Brazil nuts . .	1 oz. =	2	5	20	208
Pickles (mixed, unsweetened) . .	1 oz. =	1·2	0·2	0·1	6·5
Potato . . .	1 oz. =	5	0·5	0	22
Gruyère cheese . .	1 oz. =	0	10	10	130
Gorgonzola cheese .	1 oz. =	0	7·5	9	111
Stilton cheese . .	1 oz. =	0	7·5	10·5	134·5
Camembert cheese .	1 oz. =	0	6·6	6·6	85·8
Chicken (roast or boiled) . .	1 oz. =	0	7·5	2·2	49
Stewed rabbit . .	1 oz. =	0	10	2	58
Sweetbreads (cooked) .	1 oz. =	0	5	0·5	24·5
Ox tongue (tinned) .	1 oz. =	0	6·6	8·6	103·8
Olive oil . . .	1 oz. =	0	0	30	270

Thus the different varieties of cheese may be interchanged, as there is very slight difference in their composition, and ½ oz. brazil nuts may be substituted for ½ oz. of butter. Chicken or rabbit may be eaten instead of lean meat, or twice their weight of sweetbreads. Ox tongue contains more fat than lean meat, but may usually be substituted for it. Mint sauce may be made from mint, saccharin and vinegar. Fish is best taken boiled, and may be flavoured with a small quantity of anchovy or Worcester sauce. Just under half an ounce of olive oil may be taken instead of the butter allowance, and used together with a little cream and vinegar as a salad dressing, the salad being made from the egg and vegetables A.

Tea.—A little sugarless jam, such as that made by Callard, or special diabetic biscuits, as Callard's kalari biscuits or batons, may be taken as an alternative to the standard meal,

or the bread may be made more attractive by a small quantity of fish paste or gentleman's relish.

Dinner.—Variations may be made in the meat, chicken, or fish, as for lunch. The carbohydrate may also be alternated, as by taking 2½ oz. of potato instead of 1 oz. of bread.

Anæsthetics in Diabetes

Anæsthetics may be arranged in the following order from the point of view of safety in cases of diabetes :—

1. Local,
2. Spinal,
3. Gas and oxygen,
4. Ether, and
5. Chloroform.

The latter should never be used. In emergency cases, where a general anæsthetic is required and there is no time to render the patient sugar free or to abolish ketosis, the following procedure should be adopted.

The patient must not be starved before the anæsthetic. Just before the anæsthetic, give 30 units of insulin, and 1½ oz. of glucose (42 g.) in water by mouth. After the operation, put the patient for the first few days on the ordinary milk diet, and 20 units of insulin six-hourly, testing the urine every six hours. Larger doses, however, may be required. If a specimen become sugar free, give no more insulin until sugar reappears, and then give 10 units six-hourly. If there be no emergency, the operation should be postponed until the patient has been rendered sugar free and is on a fairly adequate dietary. If frequent blood sugar estimations be available, the correct insulin dosage is determined by them rather than by urine examinations.

Diagnosis and Treatment of Coma in Diabetes

Coma occurring in the course of diabetes may be due to (1) ketosis or (2) hypoglycæmia. There may be some

difficulty in differentiating between these two conditions, but before considering the differential diagnosis it would be well to describe them.

Coma due to Ketosis.—The best way of describing this subject is in a manner approaching that in which the practitioner or house physician would meet it. He is summoned to the bedside of a person lying unconscious, and either through a previous history of diabetes, or through a careful clinical and biochemical investigation, he comes to the conclusion that a diagnosis of diabetic coma is probable. The importance of accurate diagnosis cannot be too highly stressed, but it is obviously impossible to consider the differential diagnosis of coma in an account such as this. From the clinical point of view one might state briefly that the patient in diabetic coma will show air hunger, the breath will smell of acetone, and this is particularly noticeable if the patient be in bed in a small room ; all the muscles are equally flaccid, the deep reflexes are depressed and the plantar responses are flexor ; in 65% of cases the pulse rate is over 130 per minute ; there is also often a leucocytosis of from 16,000 to 60,000 white cells, even apart from any recognisable infective conditions (Joslin (38)). Another point which is considered as being of great diagnostic importance is the softness of the eyeballs ; this may readily be determined on palpation and is a constant feature.

The importance of a thorough general examination is great, and there can be no doubt that one of the most helpful signs in diagnosis is the temperature. In a case of straightforward diabetic coma this is always sub-normal, and it is a safe rule to work on that, if the temperature be raised or be even normal, then the coma is complicated by some second factor or is due to some other condition such as meningitis. Thus, examination of the chest may show that the patient has advanced pulmonary tuberculosis, a fact often discovered at the post-mortem for the first time ; or it may be that some obvious septic infection, such as gangrene of the extremities

or a carbuncle, is present. The discovery of any of these is of great importance, since it will indicate to the physician that he will probably have to use much larger doses of insulin than in an ordinary case, and he need not fear to anything like the same extent the onset of hypoglycæmia. If a superficial septic infection be found, it is advisable to treat this at once in the usual manner, and to render it as clean as possible. Many physicians are of the opinion that some form of septic focus will always be found in diabetic coma.

One final word of advice may be given about the possibility of mistaking cerebral lesions such as meningitis or cerebral hæmorrhage for diabetic coma. Meningitis and cerebral hæmorrhage are associated with hyperglycæmia and glycosuria, and a certain amount of ketosis may be present. They are excluded on a careful clinical examination. The possibility should always be borne in mind of encountering a diabetic patient with coma from some other cause, such as hemiplegia or cerebral hæmorrhage. The subject of hypoglycæmic coma will be dealt with later.

The next step is to obtain specimens of blood and urine, the latter by passing a catheter. The blood should be sent to the laboratory for estimation of blood sugar, blood urea and alkali reserve, the latter being of great value from a prognostic point of view, since it is a more delicate indication of ketosis than the simpler tests of Rothera and Gerhardt. It will usually be found that the urine contains large quantities of sugar, turning Benedict's reagent a deep orange-red colour, and the Rothera and ferric chloride tests will be strongly positive ; on rare occasions, however, sugar is not present. The urine should also be examined for albumin and for casts, and the amount passed every twenty-four hours measured.

The statement that diabetic coma can be found in a patient who is sugar free may be read in most text-books, yet no account of how this occurs is given. If the patient be sugar free, how may the diagnosis of diabetes be made ? We have seen one case of coma under these conditions.

The patient had glucose in the urine and was suddenly deprived of carbohydrate in his food. His urinary sugar disappeared, but the fat metabolism became so deranged that he went into coma.

There is another rare group of cases where the urine of the comatose patient contains no acetone bodies ; further, in some of these the breath may smell of acetone and acetone bodies be found in the blood and cerebro-spinal fluid. In this type of case the volume of urine passed in the twenty-four hours is generally low, and casts and albumin are present. The blood urea is also usually raised. Cases approximating to this type have been recorded by several observers, including Begg (39), Payne and Poulton (40), Oliver (41) and Graham (42) in England, and Rosenbloom (43), Joslin (44) and Paddock (45) in America.

Without waiting for the blood sugar result, 40 to 60 units of insulin should be given subcutaneously at once, together with 2 oz. (56 g.) glucose dissolved in 1 to 2 pints (568 to 1,136 c.c.) of water by mouth or administered through a stomach tube if the patient cannot swallow. Some observers have recommended the administration of insulin by the intravenous route, maintaining that if the circulation be very depressed or the blood very viscous, a more immediate effect will be obtained. Whilst it may be desirable to adopt this procedure in very severe cases owing to a great deal of collapse, our experience has shown that the intravenous administration has no advantage over the subcutaneous method. Whilst sodium bicarbonate used to be given in large quantities, it has recently been found that just as good results are generally obtained by using insulin and glucose alone. Starr and Fitz (46) have, however, shown that in some cases the acidosis may be due in part to acid substances other than ketone bodies, which are not affected by insulin. As a routine, therefore, it is advisable to administer sodium bicarbonate g. 15 (4 dr.) by mouth or stomach tube every twenty-four hours. The glucose should be administered in

as large a quantity of water as is conveniently possible, as this tends to combat the dehydration of the blood, as shown by the so-called " sticky " blood described by Graham (42) and Kennedy (47). The patient is usually dehydrated by the polyuria and 8 oz. of water should be given by mouth or stomach tube every hour. If the circulation be very depressed, as shown by a low blood pressure, it would be advisable to give the glucose intravenously in order to avoid the delay in absorption. In this case 20 c.c. of a 10% solution (=2 g. or ½ dr. glucose) in normal saline should be given into a vein.

Whilst these investigations are being conducted, the report on the blood sugar should be at hand, although, of course, in private practice this may not be possible. If the patient be in very deep coma, the blood sugar will probably be very high—in the region of 500 mg. per 100 c.c., whilst the alkali reserve will be greatly reduced, possibly to a figure below 30 c.c. CO_2 per cent. In such a case it would be advisable to give another 50 units of insulin immediately, together with another 2 or 3 oz. of glucose in the manner already described. If, however, the patient be only lightly in coma, the blood sugar being only moderately raised and the alkali reserve over 35 c.c. CO_2 per cent., then it will not be necessary to give more insulin at once. If blood sugar estimations be not available, then urine specimens, taken every three hours just before the insulin is injected, will serve as a control, and no further insulin should be given until the first three hours have elapsed.

There will now be a period of three hours before any more specific anti-diabetic steps can be taken, and this period should be employed in attending to the patient's general condition. If the patient be cold and collapsed, he should be kept warm either by means of hot water bottles, great care being taken that these are not so hot as to burn the unconscious person, or by means of a cradle fitted with electric lights. If the latter be employed, it is essential

to keep a careful watch on the temperature in the bed. It should not be allowed to rise above 99° to 100° F., and one light will usually be found sufficient.

The blood pressure has already been referred to; if this be low, it indicates that the patient is suffering from severe shock and is in imminent danger of dying from circulatory failure. Thus, a blood pressure of below 70 mm. Hg systolic points to a very grave prognosis, and steps should be taken immediately to combat this side of the disease. There are several means at the disposal of the physician: unless the blood is "sticky," a blood transfusion may be given, and a pint of blood of the right group introduced into the patient's circulation; or a pint of warm normal saline may be transfused slowly into a vein. Recently, Graham (42) has suggested that a hypertonic saline solution should be administered intravenously if the blood be "sticky"; 1 pint (568 c.c.) of a 1·2% solution of sodium chloride may be given. The increased concentration of the blood is shown roughly by the difficulty with which it is withdrawn into a syringe from a vein. More accurate indications are given by (1) the hæmoglobin percentage (see p. 397) and (2) the total solids in the blood (see p. 407). If the former be above 100%, or the latter over 25%, intravenous saline should be given and repeated in four hours' time, if still indicated.

It has been claimed by some observers that the underlying cause of circulatory failure is loss of capillary tone, thus leading to stagnation in the capillaries, followed by concentration of the blood. To counteract this ephedrine hydrochloride, gr. $\frac{1}{2}$ in 1 c.c. ampoule, may be given hypodermically; in some cases pituitrin, $\frac{1}{2}$ c.c. at two-hourly intervals, has been employed. The disadvantage of the latter procedure is that the blood sugar is immediately raised owing to stimulation of the glycogenolytic functions of the liver. In the case of cardiac failure, digitalin, gr. 1/100, or strophanthin, gr. 1/250, may be given intravenously, or

strychnine, gr. 1/60, camphor, gr. iii, or caffeine sodium salicylate, gr. ii, subcutaneously.

Attention should be paid to the bowels, as patients in diabetic coma are often very constipated. It will usually be found necessary to administer an enema followed by washing out of the colon, this being effected by moving the patient on to his side. A word of warning should be given about administering enemas to very collapsed and weak patients. If the case be a very severe one, it will probably be wiser to postpone any vigorous measures of this type until the patient has recovered consciousness. If the results of the enema have been satisfactory, ½ pint (284 c.c.) of normal saline should be introduced into the rectum in the hope that it will be retained and absorbed. Some workers have recommended a rectal injection of hot coffee and have claimed that this has remarkable effects in stimulating the patient.

By this time the first three hours following the injection of insulin will be at an end. If the patient has only been lightly in coma, it is possible that consciousness will have returned. This takes place in a manner very similar to a patient recovering from an anæsthetic. The respiration changes from the regular, long, sighing type to the irregular, noisy variety, the patient grunting at first, then groaning, and often shouting in a drunken manner. The limbs, after being in a state of extreme flaccidity, begin to stiffen and the arms are waved about, and gradually the patient becomes rational.

In any case, the bladder should be emptied, either by catheter, or if possible by the patient's voluntary action, and the urine tested for glucose and acetone bodies. If possible, blood should be taken and the sugar content and alkali reserve determined. If the patient have recovered consciousness or show signs of doing so, and there be still sugar and acetone bodies in the urine, then 25 units of insulin may be given with 1 oz. of glucose in 1 to 2 pints of water; but if the patient be still deep in coma and the

urine loaded with sugar and acetone bodies, then a larger dose must be given. If it be possible to obtain the blood sugar figure and this be high, then 100 units of insulin may be given subcutaneously with proportionately larger doses of glucose. Variations in the severity of the condition require gradation of the dosage between these limits. If the blood sugar show little or no change from that on admission, and the patient be still deep in coma, 150 to 200 units should be given. It is unwise, however, to give such large quantities unless an accurate watch can be kept on the blood sugar.

This type of regimen is carried out until the patient recovers consciousness, specimens of urine being examined every three hours just before the next injection of insulin and the glucose are given. In very severe cases it may be necessary to give as much as 2,000 units of insulin during the twenty-four hours, but with these large doses the physician must be for ever on the watch for a sudden fall in the blood sugar with the concomitant production of hypoglycæmic coma. If blood sugar estimations be not available, not more than 10 to 20 units of insulin should be given if the urine contains only a little sugar, as it is possible for the blood sugar to fall to 70 mg. per 100 c.c. or less and the urine still to contain some sugar. It is very important, however, not to be too overawed with this danger, as it must be realised that many more patients die from unrelieved hyperglycæmia than from hypoglycæmia.

When consciousness has been regained and the patient is quite rational, he has to be treated with just as much care and thought as when in coma. It is possible that the urine may be sugar free and the blood sugar normal, and it is a common error to let the patient have a twelve hours' fast from this time onwards, it being the intention to give him a No. 1 diet on the following day. The observer is then often astonished to find that at the end of a twelve hours' fast the urine is loaded with sugar and acetone bodies and the

blood sugar is up to some 400 or 500 mg. per 100 c.c. The reason for this is not difficult to see when one considers what has been happening during the period of treatment. The patient who has just come out of coma and is sugar free is by no means in the same condition from the metabolic point of view as the non-comatose person who has been starved until he is sugar free. This difference can readily be demonstrated by letting them both starve for twelve hours. Whilst the non-comatose case will remain sugar free, the treated coma patient will start to pour out sugar into his blood and urine.

The reason for this is that the patient with coma has been given enormous doses of insulin combined with glucose, with the result that the glycogenetic function of the liver already referred to at the beginning of this chapter has been stimulated and the liver has become packed with glycogen. At the end of the period of treatment, when consciousness has been regained and possibly the urine is sugar free and the blood sugar normal, the liver is loaded with glycogen and the diabetic condition only temporarily under control. Now if such a patient starve for twelve hours and receive no insulin, the liver discharges its glycogen in the form of glucose and the blood sugar immediately goes up and sugar appears in large quantities in the urine. In addition to the return of glycosuria, acetone bodies appear in high concentration in the urine, and the alkali reserve falls. If this be allowed to continue the patient will relapse into coma again. These results can only be explained by the fact that the diabetic condition becomes re-established and the system becomes flooded with carbohydrate which cannot be metabolised, owing to lack of insulin.

In the case of the non-comatose diabetic who has been starved until he is sugar free, the liver contains no glycogen, and consequently further starvation produces no change in the blood sugar. It follows, therefore, that even if consciousness has been regained in the comatose diabetic, it is

necessary to continue the insulin in order to prevent the glycogen already deposited in the liver from being discharged into the blood stream.

The ideal course of events would be to put the patient on about a No. 7 diet, but it must be realised that the events of the last twenty-four hours have been a severe strain on the patient and he is not in a fit state, mentally or physically, to commence eating ordinary food, although he may be hungry. By far the best thing to do is to give the patient ½ pint of milk every three hours, and to give enough insulin either to keep the urine sugar free or, in the event of being unable to obtain blood sugar estimations, with just a trace of sugar in the urine. After twenty-four to twenty-eight hours the patient's strength will have recovered, and he may be given a diet such as a No. 5, 6 or 7, according to his general condition, appetite, etc.

As soon as this diet is instituted, ketone bodies will almost certainly appear in the urine, and frequently the Rothera and ferric chloride tests will give a very strong response indeed, quite sufficient in fact to cause the greatest alarm to those unaccustomed to managing this stage of the treatment. But experience has shown that there is no need to fear the onset of coma, and the best method of dealing with the situation is to add 2 to 4 dr. of glucose three times a day to the diet. This will usually be found sufficient to abolish the ketosis, and as time goes on this may gradually be reduced as the diets are worked up.

It is still advisable to continue collecting the urine in three-hourly specimens, commencing at 6 a.m. and omitting the 3 a.m. collection in order to allow the patient six hours' undisturbed rest. By this means it will be possible to find out at what period of the day sugar is secreted and distributed, and the insulin can then be given at the time it is most required.

From this period onwards the treatment of the diabetic follows the ordinary lines laid down, the extra glucose being

omitted from the diet as the acetone bodies disappear from the urine, and the diets gradually increased through the series until the basal requirement diet has been reached. As soon as the patient is on the progressive series of diets and the stage of immediate anxiety is passed, the physician should institute a very thorough search for a septic focus. This will include the ordinary methods of examination, and in addition such special methods as X-rays of teeth, sinuses, examination of tonsils, appendix, and gall-bladder.

It is essential to commence treatment, both insulin and general, at as early a moment as possible, as experience has shown that the longer the patient remains in coma untreated the more difficult is it to revive him. If, therefore, the means for investigation of blood are not at hand, 50 units of insulin with 2 oz. of glucose should be given at once.

The importance of albuminuria in diabetic coma, with concomitant increase in the blood urea and non-protein nitrogen, has been emphasised by many workers. It is stated by some that if the blood urea be very high then the prognosis is bad. In our experience, however, it is very common to find a blood urea of 70 mg. per 100 c.c. or over, in ordinary, straightforward diabetic coma, and in our opinion this is not necessarily of grave prognosis. A combined nephritic type of case is recorded as described on p. 122, but this is usually associated with suppression of urine, albuminuria, and granular casts in the urine, in which case the outlook is very grave.

Coma Due to Hypoglycæmia.—This condition may arise during the treatment of a diabetic, either due to an overdose of insulin, or to the patient not eating his diet and still continuing the insulin, or to his taking too much exercise. Sometimes, during the treatment of coma, the patient may pass from hyperglycæmic coma to hypoglycæmic coma without recovering consciousness. This course of events can occur if the blood and urine sugar are not watched very carefully in the manner described. The danger of this

happening is the justification for giving glucose with the insulin, as this acts as a buffer against hypoglycæmia, and in our opinion large doses of insulin should never be given to an unconscious patient without a simultaneous administration of glucose by a stomach tube, or intravenously. It has been observed by Hart and Bond (48) that in hypoglycæmic coma an extensor plantar response may occur, whereas this appears never to be so in coma due to ketosis. This may serve as a helpful point in differential diagnosis. Should hypoglycæmic coma occur, an injection of 1 c.c. of pituitrin should be given subcutaneously, followed, if necessary, by the intravenous administration of 20 c.c. of a 10% solution of glucose (=2 g. or ½ dr. glucose) in normal saline.

Early Symptoms of Hypoglycæmia

Usually these do not appear until the blood sugar falls to 50 mg. per 100 c.c. Considerable variations from this may occur. Thus, with patients who for long have become accustomed to a high blood sugar content, hypoglycæmic symptoms may appear when the sugar has only fallen to 250 mg., as recorded by Leyton (37), or to 150 mg. per 100 c.c., as in the case quoted by Langdon Brown (29).

The condition is, perhaps, analogous to the state of the patient who has become accustomed to a high blood pressure, and is uncomfortable if it be lowered, although only to a figure considerably above the normal for a healthy person of the same age.

On the other hand, hypoglycæmic symptoms may not be noticed until the blood sugar falls to 32 mg. per 100 c.c. (Banting (31)).

The early symptoms are variable and include sweating, apprehension, flushing, pallor, a sense of constriction around the waist, and coldness of the extremities. These manifestations may be followed by collapse, convulsions and deli-

rium. The symptoms usually first appear about two hours after the injection of insulin, but may not be noticed for as long as twelve hours. At the earliest warning the patient should take two lumps of sugar or a few sticks of barley sugar. An injection of 1 c.c. of 1/1,000 adrenalin chloride, or 1 c.c. of pituitrin intramuscularly, is of value if there remain any glycogen stored in the liver or muscles. Pituitrin appears more valuable in this respect than adrenalin, as it has an antagonistic action to insulin, apart from the glycogen content of the liver. As a prophylactic measure it is important to remember that an adequate carbohydrate meal must always be given at night after the evening dose of insulin in order to prevent hypoglycæmia developing during sleep.

REFERENCES

1. Banting and Best.
Journ. Lab. Clin. Med., 1921–22, vii., 264; *Trans. Roy. Soc. Canada*, 1922, xvi., 6.
2. Collip.
Journ. Biol. Chem., 1923, lv., 462.
3. Doisy, Somogyi and Shaffer.
Journ. Biol. Chem., 1923, lv.; *Scientific Proceedings*, p. xxxi.
4. Dudley.
Biochem. Journ., 1923, xvii., 376.
5. Dudley and Starling.
Biochem. Journ., 1924, xviii., 147.
6. Dodds and Dickens.
Lancet, 1924, i., 330, and *Brit. Journ. Exp. Path.*, 1924, v., 115.
7. Burn.
Journ. Physiol., 1923, lvii., 318.
8. Best, Smith and Scott.
Amer. Journ. Physiol., 1924, lxviii., 161.
9. Baker, Dickens and Dodds.
Brit. Journ. Exp. Path., 1924, v., 327.
10. Dodds and Dickens.
"*The Chemical and Physiological Properties of the Internal Secretions.*" 1925. Oxford Univ. Press.
Macleod and others.
Physiol. Reviews, 1924, iv., 21.

11. Taylor and Hulton.
Journ. Biol. Chem., 1916, xxv., 173.
12. MacLean.
"*Modern Methods in the Diagnosis and Treatment of Glycosuria and Diabetes*," 1924. 3rd ed. Constable, London.
13. Bennett and Dodds.
Lancet, 1925, i., 429.
14. Cammidge.
"*The Insulin Treatment of Diabetes Mellitus*," 1924. Livingstone, London.
15. Bensley and Lane.
Amer. Journ. Anat., 1911–12, xii., 297.
ibid., 1916, xix., 37.
16. Kennaway.
Guy's Hospital Reports, 1913, lxvii., 161.
17. Van Slyke.
Journ. Biol. Chem., 1917, xxxii., 455.
18. Bence-Jones.
Quoted in Schafer's "*Text-Book of Physiology*," 1898, i., 410. Pentland, Edinburgh.
19. Haldane.
"*Methods of Air Analysis*," 1918. Griffin & Co., London.
20. Dodds.
Journ. Physiol., 1921, liv., 342.
21. Hurtley and Trevan.
Journ. Physiol., 1916, i., xlix.
22. Van Slyke.
Journ. Biol. Chem., 1917, xxx., 347.
23. Haldane and Priestley.
Journ. Physiol., 1905, xxxii., 225.
24. Poulton.
Brit. Med. Journ., 1915, ii., 392.
25. Fridericia.
Hosp. Stidende., 1914, lvii., 585; also
Fridericia and Olsen.
Deutsch. klin. Med., 1912, cvii., 236.
26. Allen.
"*Studies concerning Glycosuria and Diabetes*," 1913. Harvard Univ. Press, Cambridge, Mass.
27. Guelpa.
Brit. Med. Journ., 1910, ii., 1050.
28. Graham.
Quart. Journ. Med., 1917, x., 245.
29. Brown.
Lancet, 1924, i., 59.
30. Woodyatt.
Arch. Int. Med., 1921, xxviii., 125.

31. Banting.
Brit. Med. Journ., 1923, ii., 446.
32. Murray Lyon, Robson and White.
Brit. Med. Journ., 1925, i., 207.
33. Frank, Nothmann and Wagner.
Deutsch. Med. Woch., 1926, lii., 2067 and 2107.
Klin. Woch., 1926, v., 2100.
34. *Med. Res. Counc. Rep.*
Lancet, 1927, ii., 517.
35. Dreyer.
"*Assessment of Physical Fitness*," 1920. Cassell, London.
36. Graham.
Lancet, 1924, i., 63.
37. Leyton.
Lancet, 1923, ii., 1125.
38. Joslin.
"*The Treatment of Diabetes Mellitus*," 1928, 653. 4th ed. Lea and Febigher, Philadelphia.
39. Begg.
Lancet, 1925, ii., 69.
40. Payne and Poulton.
Lancet, 1925, ii., 68.
41. Oliver.
Lancet, 1926, i., 750.
42. Graham.
Bart's. Hosp. Reports, 1929, lxii., 55.
43. Rosenbloom.
New York Med. Journ., 1915, cii., 294.
44. Joslin.
"*The Treatment of Diabetes Mellitus*," 1928, 213. 4th ed. Lea and Febigher, Philadelphia.
45. Paddock.
Journ. Amer. Med. Assocn., 1924, lxxxii., 1855.
46. Star and Fitz.
Arch. Int. Med., 1924, xxxiii., 97.
47. Kennedy.
Lancet, 1928, i., 660.
48. Hart and Bond.
Brit. Med. Journ., 1929, i., 895.

CHAPTER IV

PANCREATIC FUNCTION

The investigations of pancreatic function can be divided into two groups, according to whether the internal or external secretory process is to be examined. The chief disorders of internal secretion have been fully dealt with in the chapter on glycosuria, consequently it is only necessary to consider here the investigation of pancreatic function as a whole, and also briefly to mention the pressure effects and nervous symptoms associated with lesions of the gland.

The pancreatic secretion itself contains the most important digestive enzymes in the body. They are :—

Trypsinogen, which, although inert in itself, is activated by the enterokinase of the succus entericus on reaching the intestine. This enzyme is capable of reducing most proteins to amino-acids. The infantile pancreatic juice is stated to contain no trypsinogen until about the sixth month.

Rennin, which acts upon the calcium caseinogenate of milk, converting it finally into the insoluble calcium caseate, which is digested by erepsin.

Lipase or *steapsin*, a powerful enzyme, splitting fats into fatty acids and glycerin.

Diastase, which hydrolyses starch into maltose and dextrin ; and probably some *invertases*.

The pancreatic ferments may be regarded as finishing off the work of the stomach. They deal with every group of foodstuff, and consequently any derangement of the external secretion should produce definite alterations in digestion and absorption.

Thus complete absence of the secretion should be attended with :—

Presence of undigested protein in the fæces, *i.e.*, azotorrhœa or creatorrhœa.

Presence of excessive quantities of fat, or steatorrhœa.

Presence of free starch in stools.

Examination for any of these three conditions will constitute a test for pancreatic efficiency.

Demonstration of Inefficient Protein Digestion

This can be done by examination of the fæces for undigested muscle fibres. The microscopic examination of fæces is considered by many, and more particularly by continental workers, as being of the greatest value in the diagnosis of pancreatic insufficiency.

The method of examination consists in preparing two slides, one a direct smear of the fæces, and the second made by taking a small quantity of the fæces on the end of a glass rod and rubbing it up in a few c.c. of water to give an opalescent fluid; this is centrifuged and the deposit put on the second slide for examination. It may be desirable to take a third slide, staining it with carmine. In the first slide the observer will look for globules of fat or gross, undigested particles. If there be deficiency of the external pancreatic secretion, oily drops will be seen and large fragments of food and vegetable matter. If the case be one showing biliary obstruction, fatty acid crystals will be apparent. In the centrifuged specimen, muscle fibres will be seen, and if there be inefficient digestion, these will be found in large numbers and the striations will be readily detectable. Normally a few muscle fibres may be seen in fæces, but on these the striations will be very faint or completely absent. Great care must be taken not to confuse the long, reticular scalariform vessels of vegetable substance with striated muscle fibres. Fibrin in the form of spirals may occasionally be seen together with large masses of undigested material.

It must of course be pointed out that the only way for the untrained observer to learn the value of microscopic examination of either fæces or urinary deposits is by having some typical slides explained to him by a skilled worker. Reference to descriptions or even illustrations is of little value.

It is as well to stain the smear with carmine prior to the microscopic examination. The test may be carried out more scientifically by using a standard test diet, such as that of Schmidt (1), which consists of the following :—

Breakfast.

½ litre milk.
50 g. biscuit or bread.

Lunch.

125 g. chopped beef, cooked so that the interior is raw.
250 g. potatoes.
100 c.c. milk.
10 g. butter.

Tea and *Dinner*, the same as breakfast and lunch.

A charcoal biscuit may be given before and at the termination of the diet. The fæces are collected in between the appearances of the charcoal.

The stools are examined microscopically, as detailed above. One slide may, in addition, be stained with Lugol's iodine, and the presence or absence of large numbers of starch granules noted. All observers agree that the bulkiness of the stools is of the greatest importance in the diagnosis of pancreatic lesions.

Schmidt also laid great stress on the presence of nuclei in the fæcal *débris*, since he stated that the nuclei of animal cells were digested by the pancreatic secretions alone.

Demonstration of Inefficient Fat Digestion

Normally dried fæces contain up to 25% by weight of fat. About 7 to 12% of the ingested fat passes out by the

fæces. Deficient external pancreatic secretion leads to an increase in the fæcal fat content, this increase being in the neutral fat fraction of the total. The fæcal fat content may be determined in a number of ways, but Cammidge's (2) adaptation of the Schmidt-Werner process is in general use.

A small quantity of fæces is dried to constant weight by means of a boiling water bath and a vacuum desiccator. The residue is ground up to a fine powder with a pestle in the dish, care being taken to ensure that any fat which has melted and run up the side of the dish is thoroughly mixed with the residue. 0·5 g. is weighed out and introduced into a Schmidt-Werner milk tube marked A. Ten c.c. of 30% HCl are added, and the tube is heated in a boiling water bath for a quarter of an hour. It is as well to agitate the tube every few minutes, but the greatest care must be taken throughout the whole process that no particles adhere to the sides of the tube above the acid level. After cooling, the tube is filled to the mark with ether, and is securely corked. The tube is then inverted at least fifty times in such a manner that the distribution of the aqueous and ethereal layers is reversed. It is then stood in an upright position, and is rotated to facilitate complete separation of the layers. When the supernatant liquid is clear, 20 c.c. are pipetted off into a weighed vessel, which is then heated to evaporate off the ether. When dry, the vessel is placed in a vacuum desiccator and afterwards weighed. The amount of ether extract in 20 c.c. can then be calculated.

The height of the remaining column of fluid in the Schmidt-Werner tube should be read off.

The percentage of fat in the fæces can then be calculated by the following method :—

If w = weight of fæces taken,

x = the weight of fat found in 20 c.c. of ether,

y = the number of c.c. of liquid left in the tube.

Since 10 c.c. of 30% HCl were contained in the tube, the

amount of ether left will be $(y - 10)$ c.c. The total amount of ether throughout which the fat was distributed will be

$$20 + (y - 10) \text{ c.c., } i.e., y + 10 \text{ c.c.}$$

$(y + 10)$ c.c. therefore represents the total volume of ether, throughout which the fat was distributed in the proportion of x g. per 20 c.c. The fat content of $(y + 10)$ c.c. will therefore be $\frac{x\ (y + 10)}{20}$ g. This gives the fat content of w g. of fæces. The percentage will therefore be given by the following calculation :—

$$\frac{x\ (y + 10) \times 100}{20 \times w}.$$

It is now necessary to determine the relative amounts of fatty acids and neutral fats. This can be done by weighing out a fresh 0·5 g. of the dried fæces into a tube marked B, and proceeding as above, except that 10 c.c. of distilled water are used for the first extraction instead of 30% HCl. Twenty c.c. of the ether extract are pipetted off, and after evaporation the dry residue is weighed. This is dissolved in about 20 c.c. of ether, a few drops of phenolphthalein in alcohol are added, and the solution is titrated with $\frac{N}{10}$ alcoholic NaOH. It is usual to calculate the result in terms of stearic acid, although it must be understood that such a procedure is quite arbitrary. The molecular weight of stearic acid is 284, therefore 1 c.c. of a decinormal solution would contain 0·0284 g. If, therefore, the number of c.c. of $\frac{N}{10}$ alcoholic NaOH used in the neutralisation be multiplied by 0·0284, the fatty acid content of the 20 c.c. of ether pipetted off will be expressed in grams of stearic acid. This figure, multiplied by two, will give the fatty acid content of the weight of fæces originally taken. The weight of the ether residue is then calculated as a percentage of the dried fæces. This represents the unsapo-

nified fat, or, as Cammidge calls it, the "unsoaped fat." If the fatty acid content be expressed as a percentage, and subtracted from the above, the free neutral fat content can be arrived at.

Cammidge, who evolved these methods, states that he has performed some thousands of analyses on these lines with very satisfactory results.

The methods are very rapid and simple. Cammidge gives the following as normal figures :—

Total fat	= 15 to 25%	by weight of dried fæces.
"Unsoaped" fat, *i.e.*, total weight of ether soluble material obtained in B .	= 10 to 15%	,,
Neutral fat	= 1 to 2%	,,
Free fatty acid . . .	= 9 to 13%	,,
Combined fatty acids . .	= 10 to 15%	,,

In pancreatic insufficiency the total fat is increased up to 60, 70 or 80%, and this increase is found to be in the neutral fats, thus showing defective fat splitting. In obstructive jaundice the total fat is also increased, but, since the fat-splitting action is normal, the increase is in the fatty acid fraction. Thus a defect of digestion (pancreatic lesion) is associated with a high neutral fat content, whilst with a defect of absorption (obstructive jaundice) the increase is mainly in the fatty acids.

Investigation of the duodenal contents is described in Chapter V.

Loewi's Test (3)

This observer noted an increase in irritability of the sympathetic nervous system after removal of the pancreas. This could be demonstrated by the fact that adrenalin would dilate the pupil, a reaction not seen in normal animals. This reaction was applied clinically with some success. Two drops

of 1/1,000 adrenalin are instilled into the conjunctiva, and the pupil is examined a quarter of an hour after. If still undilated, a further 2 drops are instilled, and the pupil is examined again later. If dilatation be present, a pancreatic lesion is suspected. Although this test must depend upon some obscure internal secreting action of the pancreas, very good results have been recorded. Thus Sladden (4) states that the test is very reliable. Cases showing hyperthyroidism are also said to give this reaction.

The Diastase Test

Wohlgemuth (5) was the first to show that urine contains a starch-splitting enzyme, and to evolve a method for its estimation. A unit of diastase, according to Wohlgemuth, is given by the number of c.c. of 0·1% starch solution digested by 1 c.c. of urine. Normally, the diastatic index of urine falls between 6·6 and 30. Its presence in the urine is usually explained by the fact that diastase is absorbed from the alimentary canal into the blood stream, from which it is excreted by the kidneys into the urine. Although the pancreas is held to be the site of formation, it has been stated that the urinary diastase is increased after pancreatectomy, and, consequently, some other organ has been regarded as the source, more particularly the liver. Despite the confusion as to its origin, elevations or depressions in the urinary diastatic index form very useful guides to diagnosis. In renal disease, the index is usually below 6·6, owing to the fact that diastase, being a colloid, is excreted with difficulty. The use of this reaction as a renal function test has already been discussed (Chapter II.). In acute pancreatitis, the index rises to 100, 200, or even to higher figures. It is assumed that in these conditions there is pancreatic obstruction, with the result that diastase passes straight into the blood and thence to the urine. In chronic pancreatitis the index may or may not be affected. Various other conditions have been stated

to cause an increase in the urinary diastase reaction, such as the toxæmias of pregnancy. These observations have not, however, met with support.

Original Wohlgemuth Technique (*modified by E. C. Dodds* (6)). *Solutions.*—A 0·2% starch solution. This is best made up by adding the weighed quantity of starch, pinch by pinch, to the requisite volume of distilled water, and stirring until an even suspension is obtained. The mixture is then slowly brought to the boil, stirring all the time. After boiling

	Test-tube No.				
	1.	2.	3.	4.	5—12.
0·2% starch solution.	1 c.c.	1 c.c.	1 c.c.	1 c.c.	1 c.c.—1 c.c.
Buffered urine.	1·5 c.c.	1 c.c.	0·5 c.c.	0·45 c.c.	0·4 c.c.—0·05 c.c.
Distilled water.	0·5 c.c.	1 c.c.	1·5 c.c.	1·55 c.c.	1·6 c.c.—1·95 c.c.

for a short time the solution becomes opalescent, when it is cooled and the volume is made up to the correct amount. By this means no troublesome gelatinous masses form and the solution is quite uniform. Preferably this solution should be made up fresh each day and should never be kept more than a few days.

Phosphate buffer solution.—This is obtained by mixing 15 c.c. of Sörensen's solution A with 85 c.c. of solution B.

Solution A is made by dissolving 11·876 g. of $Na_2HPO_4.2H_2O$ in 1 litre of boiled distilled water, the solution being kept in a paraffin-coated bottle.

Solution B is made by dissolving 9·078 g. of KH_2PO_4 in 1 litre of boiled distilled water, and is stored in a paraffin-coated bottle.

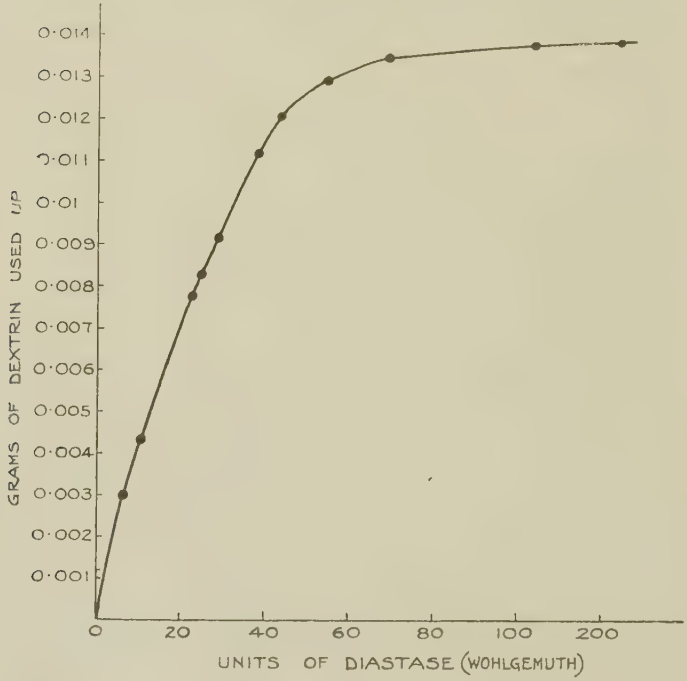
The resulting solution should have a *p*H of 6·1.

The buffer solution should be checked by the hydrogen electrode.

1·5 c.c. of urine are added to 6 c.c. of the buffer solution. The resulting solution is well shaken in order to ensure uniform distribution. A series of test tubes is then put up, each being filled as shown in table on p. 141.

Short tubes are used ($\frac{1}{2}$ inch $\times$ 4 inches), as with these

Curve to show Relation between Units of Diastase and Grams of Dextrin used up



there is less liability for drops of the various ingredients to stick to the sides of the tube, and, by dropping down when the iodine is added, spoil the end-point. The urine and the fractions of a c.c. of water are added first, followed by the starch solution. It now remains to add rapidly 1 c.c. of water to each tube except No. 1. The sides of the tube are de-

liberately washed down into the bottom with this distilled water. By adding the solutions in the above order all the tubes get about the same digestion time. The tubes are incubated for half an hour at 37° C., cooled, and $\frac{N}{50}$ iodine is added. As small a quantity as will just give a faint colour is used, excess making the end-point much more obscure.

Calculation of Results.—Suppose the tube just not showing a mauve tint, *i.e.*, the one where the starch was just digested, contained 0·5 c.c. of the diluted urine. Therefore 0·5 c.c. of diluted urine, or 0·1 of the undiluted urine, digests 1 c.c. of 0·2% starch, or 2 c.c. of 0·1% starch. Since the number of Wohlgemuth's units is given by the number of c.c. of 0·1% starch solution digested by 1 c.c. of urine, it is obvious that in the above case 20 units of diastase were present.

A colorimetric method has been devised by Cohen and one of us (E. C. D.) (7) which gives the exact number of units present.

Solutions :—

1. Buffer Solution.—As in previous method.

2. Standard Dextrin Solution.—0·75 g. of pure dextrin is dissolved in a small volume of boiled distilled water, and the volume is made up to 100 c.c.

3. Iodine Solution.—

$\frac{N}{10}$ iodine in potassium iodide solution .	20 c.c.
Saturated ammonium sulphate solution .	500 c.c.
Distilled water	to 1,000 c.c.

Method.—1·5 c.c. of urine are pipetted into a tube, and 6 c.c. of buffer solution are added. The mixture is well shaken. 0·5 c.c. of this buffered mixture is pipetted into a test tube graduated at 30 c.c., and 2 c.c. of the standard dextrin solution are added. After thorough shaking the tube is incubated in a water bath at 37° C. for half an hour. Whilst the incuba-

tion is proceeding the standards are prepared. If the approximate diastatic power of the urine be not known, it is as well to prepare three standards in order to ensure accurate comparison in the event of very high or low readings. These are made up by pipetting 1·5, 1·0, 0·5 c.c. of standard dextrin respectively into tubes graduated at 30 c.c.

When the incubation period is complete the tube is removed from the water bath, and 15 c.c. of the iodine solution are added to it and to each of the three standards. In highly coloured urine it is perhaps as well to add 0·5 c.c. of the buffered mixture to the standard immediately before comparison. The volume of each is then made up to the mark, mixed and compared in the colorimeter, the standard being set at 20 mm. The standard with the nearest depth of colour to the unknown is selected, thus avoiding errors due to too wide a difference in tint.

The amount of dextrin present in the unknown is calculated by the familiar colorimetric ratio—that is,

$$\frac{20}{x} \times \mathrm{Y};$$

where x is depth in millimetres of unknown, and Y is the quantity of dextrin in the standard. Since 2 c.c. of 0·75% dextrin solution—that is 0·015 g. of dextrin—were present before incubation, the amount of dextrin used up can be calculated by subtracting from this the figure obtained in the above calculation. By reference to the curve this can be converted into Wohlgemuth units.

Pressure Symptoms

Tumours in the head of the pancreas frequently compress the common bile duct and give rise to an obstructive jaundice. This is usually accompanied by distension of the gall-bladder, together with some secondary enlargement of the liver.

Pressure on the portal vein may also give rise to ascites, but this is a less common pressure symptom than jaundice.

Nervous Symptoms

These are most noticeable in acute inflammatory lesions of the pancreas.

They are due to irritation of the adjacent solar plexus, and include pain in the epigastrium and back, vomiting and shock.

Paroxysms of epigastric pain may also occur in the more chronic affections of the pancreas, such as new growth, or in chronic pancreatitis.

Conclusions.—It remains to evaluate the findings of tests for pancreatic efficiency. From considerable experience in routine laboratory work one would say, without doubt, that the tests are of the greatest value in the differential diagnosis of acute hæmorrhagic pancreatitis. The diagnosis of this condition from several other acute abdominal lesions is admittedly difficult on purely clinical lines, yet the estimation of urinary diastase will undoubtedly clinch the matter. It is usually stated that a diastatic index of over 100 indicates the condition, but we prefer to make a certain diagnosis only on figures of 200, although the former figure is to be regarded as very suggestive. In the records of the Courtauld Institute of Biochemistry during recent years twenty-three urines were examined of cases with symptoms typical of acute hæmorrhagic pancreatitis. Of these, nine had a diastatic index of 200 or over, and were subsequently proved either by post-mortem examination, operation or clinical progress, to be suffering from acute pancreatitis. The remaining fourteen were proved not to belong to this group, and the diastatic index was normal. We therefore place the greatest faith in this test for this purpose, although we feel that it is unwise to deduce anything from small variations. It is claimed that other conditions, such as the toxæmias of

pregnancy, are attended with a high diastatic index, but these opinions have not found general confirmation.

For chronic pancreatic lesions, the most reliable tests have been found to be microscopic examination of the fæces and the estimation of fæcal fat. In conclusion, the findings in various common pancreatic disorders are summarised in the form of a table.

Disease.	*Diastase.*	*Fæcal Fat%.*	*Muscle Fibres.*
Acute pancreatitis	200	70–90	+
Chronic pancreatitis	10–50	60	+
Neoplasm invading pancreas	30–100	40–70	+

REFERENCES

1. Schmidt.
   Quoted in "*Winternitz Diagnostikum*," 1910. Otto Stegman, Berlin.
2. Cammidge.
   "*The Fæces of Children and Adults*," 1914, 516. Wright, London and Bristol.
3. Loewi.
   Arch. Exp. Path. and Pharm., 1908, lix., 83.
4. Sladden.
   Quart. Journ. Med., 1913–14, vii., 455.
5. Wohlgemuth.
   Biochem. Zeit., 1908, ix., 1, and 1909, xxi., 432.
   Berlin. klin. Woch., 1910, xlvii., 92.
6. Dodds.
   Brit. Journ. Exp. Path., 1922, iii., 133.
7. Cohen and Dodds.
   Brit. Med. Journ., 1924, i., 618.

CHAPTER V

INVESTIGATION OF HEPATIC FUNCTION

Since the liver plays an important part in practically every metabolic process, it is impossible to commence this section with a summary of the physiology of the organ. Its bearing upon carbohydrate and fat metabolism has been alluded to in Chapter III., and in the following account the tests will be classified according to what particular function of the liver they are designed to investigate. In this manner it will be possible to obtain an insight into the physiological activities of the organ.

The actual tests have usually been classified in the following manner :—

Investigation of the pigmentary functions.

Testing blood, urine and fæces for bile pigments and their precursors.

The van den Bergh reaction.

Investigation of the metabolic functions.

The nitrogen partition methods.

The carbohydrate methods, such as the lævulose and galactose tests.

Investigation of the antitoxic powers.

The glycuronic acid test.

The indican tests.

Investigation of the relations between the liver and blood, or the so-called hæmopoietic functions.

The coagulation time of blood.

The fibrinogen content.

The blood lipase.

Widal's test. The hæmoclasic crisis.

Investigation of the global capacity of the liver to eliminate foreign substances.

The phenoltetrachlorphthalein test.

Cholecystography.

Investigation of the duodenal contents.

Lyon's test.

Investigation of the Pigmentary Functions of the Liver

It is necessary to commence with a very brief outline of the metabolism of bile pigments. Bilirubin and biliverdin are formed from hæmoglobin derived from broken down red cells. According to the older views, the liver is responsible for this production, although there is evidence to show that other tissues can effect these changes. This side of the question will be dealt with when describing the van den Bergh reaction. It will be remembered that bile pigments are converted in the intestine into a number of substances, the most important of which are urobilin and its precursor, urobilinogen.

These changes are brought about by bacteria. Urobilinogen and urobilin are absorbed into the circulation, and are picked out by the liver cells, and re-excreted into the bile as bile pigments, biliverdin and bilirubin. A very small amount of urobilin is excreted normally into the urine, and special tests are required to demonstrate its presence. Damage to the liver cells causes them to lose this property of converting urobilin into bile pigments, with the result that this body accumulates in the blood, and is excreted into the urine in easily demonstrable amounts. This change precedes the appearance of actual bile pigments in the urine by a considerable time. Thus tests for urinary urobilin are quite strongly positive in the pre-icteric stage of acute catarrhal jaundice. In three cases examined, the tests were strongly positive three days before jaundice developed. If the blood give reactions for bile, it is obvious that there must be some derangement of hepatic function.

We have also employed Fouchet's test for bile pigment in blood. The originator of this reaction claims that the test gives positive results in very high dilutions of bile-containing solutions. We have been able to confirm his statements completely, finding that the test was positive up to a dilution of 1 in 60,000.

Fouchet's Reaction for Bile Pigment in Blood (1).—To 3 drops of serum on a white porcelain surface, 3 drops of the reagent (20 c.c. H_2O, 2 c.c. of 10% $FeCl_3$, 5 g. trichloracetic acid) are added. A white coagulum results from the precipitation of the serum proteins by the trichloracetic acid contained in the reagent. If the reaction be positive the coagulum turns a greenish-blue colour, reaching a maximum in about twenty minutes.

Tests for Urobilin and Urobilinogen in Urine.—*Ehrlich's Aldehyde Reaction* (2).—To 5 c.c. of urine 2 drops of a 3% solution of paradimethylaminobenzaldehyde in 50% HCl are added. If the test be positive the resulting mixture becomes deep red. Occasionally it is necessary to warm the solution to bring about the reaction, which may take a few minutes to develop. This test, when positive, demonstrates the presence of a pathological amount of urobilinogen.

Schlesinger's (3) *Test for Urobilin.*—Twenty c.c. of urine are acidulated with acetic acid, and then the urobilin is extracted by gently inverting with 5 c.c. of amyl alcohol. The amyl alcohol layer is pipetted off and a few drops of a 10% alcoholic solution of zinc chloride or acetate are added to it. If a green fluorescence develop the test is positive ; in addition, the characteristic spectrum of the solution (a band in the green between *b* and F) can be observed. A positive reaction demonstrates an excess of urobilin.

Iodine Test for Bile Pigments in Urine.—Add a solution of iodine in KI drop by drop, and a green colour indicates the presence of bile pigment. The excess of iodine can be shaken out with chloroform.

Gmelin's Test for Bile Pigments in Urine.—Fuming

nitric acid oxidises bile pigment firstly to green, then blue, then red, and finally yellow pigment. In practice, the essential point is that the green is separated from the yellow colour by purple or reddish tinges.

If normal urine be poured on to nitric acid in a test tube, a purple or reddish colour, but no green, will result. To detect small amounts, add to a test tube of urine a few drops of 5% $CaCl_2$ solution, shake, add a few drops of 5% Na_2CO_3 solution and shake again. The $CaCO_3$ precipitate carries down pigment. Filter, scrape off most of the precipitate, and put a drop of nitric acid on to paper. A green ring is seen, the outside of which is reddish-purple if bile be present.

Tests for Bile Salts in Urine.—Hay's test consists in sprinkling flowers of sulphur on to the surface of urine in a test tube. If bile salts be present the particles sink to the bottom of the tube.

From what has been stated it is obvious that a positive Fouchet's test, or the presence of bile pigments, urobilin, or excess of salts in the urine must indicate deranged hepatic function.

Tests for Bile Pigments and their Derivatives in Fæces.—*Schmidt's Test for Hydrobilirubin.*—A small amount of fæces is rubbed up in a mortar with a concentrated aqueous solution of mercuric chloride. The mixture is transferred to a shallow, flat-bottomed dish and is allowed to stand for six to twenty-four hours. The presence of hydrobilirubin will be indicated by a deep red colour being imparted to the particles of fæces containing this pigment. This red colour is due to the formation of a hydrobilirubin-mercury compound. If unaltered bilirubin be present in any portion of the fæces, that portion will be green in colour, due to the oxidation of bilirubin to biliverdin.

Gmelin's Test for Bilirubin.—Place a few drops of concentrated nitric acid in an evaporating dish and allow a few drops of the fæces and water to mix with it. The usual colours of Gmelin's test are produced, *i.e.*, green, blue, violet,

red and yellow. This test can be performed on a slide, and observed under the microscope.

The van den Bergh Reaction.—Since this reaction is mainly concerned with the investigation of jaundice, it will, perhaps, be as well to describe the modern views on this condition, and to contrast them with the older ones. At the outset it should be stated that the reader who wishes to inquire deeply into this subject is fortunate in that he has at his disposal a number of valuable treatises. Reference to these will be found in the bibliography at the end of the chapter, but special attention should be paid to J. W. McNee's excellent article (4) in the *Quarterly Journal of Medicine*, where a full and critical review is given. In view of the easily available literature, only the briefest summary of this important work will be given here. Jaundice, at the time of Virchow, was considered to fall under two headings, hepatogenous and anhepatogenous. Thus, in the hepatogenous variety the condition resulted from obstruction of the biliary tract, whilst in the second, anhepatogenous variety, the bile pigment was thought to originate in tissues other than the liver. Later, owing to certain experimental work which need not be detailed here, the possibility of anhepatogenous jaundice was excluded. Thus Eppinger (5) stated that all jaundice was obstructive, and in those cases where obvious obstruction could not be seen the seat of the trouble was said to be in the fine bile capillaries. Later, however, the work of Aschoff (6), and his co-workers, pointed rather to the original separation of jaundice into hepatogenous, or obstructive, and hæmolytic, or anhepatogenous. His work has completely changed the views on the formation of bile pigments from hæmoglobin. Thus it used to be thought that this change was effected by the polygonal cells of the liver, but in the newer conception it is held that these simply pass the pigment on from another group of cells to the lumen of the bile capillary.

The polygonal liver cells separate the bile capillaries from

the blood stream, but between the polygonal cells and the blood stream we have a further series of cells described by Kupffer. Kupffer cells are found in the liver, spleen, and other parts of the body, and belong to what Aschoff calls the *reticulo-endothelial system*. In the modern conception, it is assumed, on certain experimental evidence, that it is in this system of cells that blood pigments are converted into bile pigments. It can thus be seen that hæmoglobin in the blood stream is converted into bile in the Kupffer cells, which normally pass it on through the polygonal cells to the lumen of the bile capillaries. The functions of the polygonal cells, therefore, are simply transmission, and they are not essentially concerned with production of pigments.

Whether actual bile pigments, or their precursors, are formed by the reticulo-endothelial system has not yet been determined. According to this theory, it is possible to have jaundice arising in one of three ways—

1. Where obstruction of the bile tract prevents the escape through the natural channels. The bilirubin so formed will then be reabsorbed into the blood stream, to be subsequently excreted in the urine.

2. Where the polygonal cells lose their power of transmitting pigment to the capillaries, with the result that it will pass direct from the Kupffer cells to the blood stream. A similar series of events might arise if, through excessive blood destruction, more pigment were offered than the polygonal cells could pass through. Some would then be absorbed direct into the blood stream, and the rest would pass normally into the biliary passages.

3. Where there is a combination of these two conditions. That is to say, although the polygonal cells are damaged, a little bilirubin might pass through into the biliary passage, from which, owing to obstruction, it would be absorbed into the blood stream. In addition to this, there would be direct absorption from the Kupffer cells.

The essential point to be realised is that, in purely obstructive jaundice, the bilirubin has passed through the polygonal cells of the liver, but that it is quite possible to have icterus due to bilirubin which has not passed through these cells. As will be shown later, these two varieties of bilirubin can be identified by a chemical reaction.

Accordingly, McNee recommends the following classification of jaundice :

Obstructive hepatic jaundice, in which there is a condition similar to Group I. in the above scheme.

Hæmolytic jaundice, corresponding roughly to Group II.

Toxic and infective hepatic jaundice, in which there is a combination of obstruction and direct absorption from the Kupffer cells similar to Group III.

In Group I., the bilirubin in the serum will be of the variety which has passed through the polygonal liver cells, whilst in Group II. it will not have been transmitted through them, but in Group III. both varieties will be present. It is obvious that if a reaction could be found to distinguish between these varieties of bilirubin, the differentiation of jaundice according to the above classification would be possible. This has been done by Hijmans van den Bergh (7), who employs Ehrlich's azo reaction. This consists in adding to the solution of bilirubin a diazonium salt in acid solution, causing the appearance of a purple compound, azo-bilirubin. The diazonium compound is provided by mixing a solution of sulphanilic acid in HCl with sodium nitrite. Van den Bergh noted that icteric sera, when treated in this manner, either gave an immediate colour, or else a very delayed reaction. He was able to assert that the first type of reaction was given by bilirubin which had passed through the liver cells, and that the delayed reaction resulted with pigment formed without the agency of the polygonal cells, *i.e.*, absorbed direct from the Kupffer cells. In cases of toxic and infective hepatic jaundice, there is usually a biphasic response, probably due to the presence of both types of bilirubin.

The following account is the revised method for performing the reaction (8): The examination must be made within about two hours of taking the blood, or paradoxical results are obtained. Ten to fifteen c.c. of blood are collected in an oxalated tube. The latter is prepared by adding 0·2 c.c. of 10% potassium oxalate solution, and evaporating to dryness during the process of sterilisation. The tube containing blood is centrifugated and the clear supernatant plasma is pipetted off.

Solutions.—(1) A freshly prepared solution of Ehrlich's diazo reagent. This is made by mixing the two following solutions immediately before the test :—

A.	Sulphanilic acid . .	1 g.
	Concentrated HCl . .	15 c.c.
	Distilled water . .	1,000 c.c.
B.	Sodium nitrite . .	0·5 g.
	Distilled water . .	100 c.c.

The actual reagent is made immediately prior to the test by mixing in the proportions of 25 c.c. of solution A to 0·75 c.c. of solution B.

(2) Absolute alcohol or 96%.

The Direct Reaction.—Three small tubes are set up (numbered 1, 2 and 3), and 0·25 c.c. of oxalated plasma is pipetted into each. To the first tube, the control, 0·2 c.c. of water is added, whilst into No. 3 a small flake of caffeine-sodium salicylate is dropped, which has been found to accelerate the development of the colour change. After the salt has been dissolved by shaking, 0·2 c.c. of fresh diazo reagent is added, when a prompt and definite reaction takes place, shown by the production of a purple tint. When this colour has attained its maximum, 0·2 c.c. of the diazo reagent is added to tube No. 2, and any alteration in colour is compared with the fully-developed reaction in tube 3.

One of three events may occur in tube 2 :—

An immediate direct reaction.—This begins at once and

reaches a maximum in ten to thirty seconds. The mixture turns to a bluish-violet colour, the intensity varying as the concentration of bilirubin.

A direct delayed reaction.—Here a reddish colour, deepening to violet, begins to appear in about fifteen minutes.

A direct biphasic reaction.—In this type of response a reddish colour appears immediately and takes much longer to deepen into a violet hue.

The Indirect Reaction.—If neither the first nor second responses be obtained the indirect reaction should be proceeded with. To 1 c.c. of plasma 0·5 c.c. of diazo reagent is added, and after a minute or so 2·5 c.c. of 96% alcohol and 1 c.c. of saturated aqueous ammonium sulphate solution are added. After thorough mixing, the tube and contents are centrifugated and the supernatant clear fluid can be used for quantitative colorimetric analysis. If the colour be too deep it may be diluted with alcohol, two parts to one of the solution.

Quantitative Reaction.—The indirect reaction may be converted into a quantitative process by matching against a standard of ferric thiocyanate in ether. This solution is made up as follows :—

Dissolve 0·1508 g. of ammonium iron alum in 50 c.c. of concentrated HCl and add water to 100 c.c. The resulting solution is quite stable and is about 1 in 320 N. To 10 c.c. of this solution add 25 c.c. of concentrated HCl, and water up to 250 c.c. This gives a dilution of 1 in 8,000 N, which keeps for six months. To 3 c.c. of this stock solution an equal volume of 20% potassium thiocyanate solution is added, and the resulting mixture is well shaken and extracted with 12 c.c. of ether. The ethereal extract is used as the standard and the solution obtained in the indirect test is matched against it. This standard corresponds to a concentration of 1 in 32,000 N of bilirubin, *i.e.*, 1 in 200,000 azobilirubin. An indirect test, containing exactly the same amount of colour as the standard, is said to contain 1 unit of bilirubin.

Van den Bergh and Müller now recommend the following as a standard solution : 2·161 g. of anhydrous cobaltous sulphate are dissolved in 100 c.c. of water, thus giving a solution which corresponds in colour with an azo-bilirubin solution of 1 in 200,000, *i.e.*, one unit. The salt must be anhydrous, and the standard solution should be checked against the one already described, *i.e.*, ferric thiocyanate, and must be stored in the dark.

Interpretation of the Results.—An immediate direct reaction indicates obstructive jaundice, whilst a delayed direct reaction points to a hæmolytic or non-obstructive form. The biphasic reaction is found in cases of toxic and infective jaundice, where both obstructive and non-obstructive processes are at work. It must be borne in mind that all sera giving a positive direct reaction also give a positive indirect reaction, although the reverse does not necessarily hold true. In a marked case of non-obstructive jaundice there may be as much as 12 units of bilirubin present in the serum. Many interesting facts have come to light since the advent of this reaction. Thus the bilirubin content of normal serum has been found to be between 1 in 1,000,000 and 1 in 400,000, or, taking 1 in 200,000 as a unit, the limits are 0·2 to 0·5 units. The renal threshold value of bilirubin has also been worked out. Thus it is stated that bile does not appear in the urine until 4 units are present in the blood. In hæmolytic jaundice this relation does not hold, since it is quite possible to have between 5 and 18 units present in the blood with no bile in the urine. It is suggested that the bile is excreted in the form of urobilin in these cases. Perhaps one of the most important developments of this reaction is the recognition of cases of latent jaundice, in which there is sufficient bile to produce slight icterus, but not sufficient to produce bile in the urine. Pernicious anæmia belongs to this group. With regard to dissociated jaundice, *i.e.*, independent retention or excretion of bile pigments and salts, little light has yet been thrown by the reaction. Hæmo-

lytic jaundice is certainly a dissociated condition, since the bile salts are unaffected. Retention of bile pigments alone has been observed in the later stages of catarrhal jaundice.

Although the van den Bergh reaction is more delicate than Fouchet's test, it is much more easily obscured by hæmolysis and similar conditions than is the latter.

It is therefore possible to have a serum giving a frankly positive Fouchet's test, but an obscured or negative van den Bergh reaction. In cases of obstruction it is very valuable to test the fæces for bile derivatives. Although the fæces are usually pale in cases of complete obstruction, the presence of blood may render the colour dark. It is in such instances that the tests for bile are of the greatest value.

The Icterus Index.—This is a useful method of following the variations in the intensity of jaundice from time to time (see p. 398).

Investigation of the Metabolic Functions

By Nitrogen Partition Methods.—Since one of the most important metabolic functions of the liver is the formation of urea from ammonia derived from the deamination of amino-acids, it might be expected that deranged function would alter the distribution of nitrogen in the various nitrogenous bodies of blood and urine. It has already been explained, in Chapter II., that the concentration of these bodies in the urine depends largely upon the amount of protein taken in the food, and, consequently, reliable results could only be expected from examination of the blood, or, in the case of the urine, by ratios. Thus the estimation of the urinary nitrogen co-efficient, *i.e.*, $\frac{\text{urea N}}{\text{total N}}$ expressed as a percentage, has been recommended as a test of hepatic function. Normally, the value of this co-efficient is between 85 and 90, whilst in hepatic inefficiency it is said to fall to 40 or 50,

indicating a decrease in the ureogenetic function. Since urea is formed by the liver from ammonia, it would be expected that in cases of deficient ureogenetic function the urinary ammonia would be increased. This can be expressed by the ammonia co-efficient (see Chapter II.), which rises as the nitrogen co-efficient falls.

On exactly similar grounds the estimation of amino-acids in blood and urine, and of blood non-protein nitrogen, have been recommended, elevation of these being said to indicate deranged function.

When the vast amount of reserve liver tissue is called to mind, and the various extraneous factors capable of modifying these figures, any test depending on the above estimations falls to the ground on general principles.

The Lævulose Tolerance Test.—The power of the liver to absorb and store large quantities of glucose has already been described. This action is not confined to glucose, but holds good also for lævulose.

Strauss (9) suggested that the estimation of lævulose tolerance might be used as a test for hepatic function. He had noticed that 90% of his cases with disorders of the liver showed lævulosuria, whilst 10% only of normal controls exhibited this phenomenon. The test consisted of giving 100 g. of lævulose by the mouth, and collecting the following twenty-four hours' specimen of urine. The sugar, if any, in this was estimated in the usual manner. In positive cases lævulose appeared in the urine about one hour after the administration of the sugar. Since the publication of Strauss' original paper in 1901, much has been written concerning this test, but the majority of observers (Wörner and Reiss (10), Churchman (11)) agree that, in its original form, the test is unreliable. Shirokauer (12) was the first to substitute the estimation of blood sugar in the place of urinary sugar. This observer noted that there was little or no rise in blood sugar content in a normal person one hour after the administration of 100 g. of lævulose. If, however, the liver

were damaged, the rise was marked—even up to 190 mg. per 100 c.c. It must be understood that the total sugar is estimated in the usual manner, and that the figure obtained represents the sum of the glucose and lævulose contents.

The patient is bled and 100 g. of lævulose are given dissolved in water. According to Spence and Brett (13), the dose of lævulose should vary with the patient's weight, according to the following figures :—

80 kg.	50 g.
60 kg.	40 g.
40 kg.	30 g.

Specimens of blood are taken at half-hourly intervals, and the urine is collected half-hourly, and is tested for sugar. If the blood sugar rise above 140 mg. per 100 c.c., or if the rise above the first value be over 30 mg., a degree of hepatic inefficiency is indicated. The urinary findings are of doubtful value.

The Galactose Tolerance Test.—Normally, the ingestion of 40 g. of galactose results in no appreciable increase of the blood sugar and little if any galactosuria. But in certain cases of deranged liver function, such as catarrhal jaundice and other maladies affecting the liver cell itself, a hyperglycæmia results and galactose is found in the urine.

In obstructive and hæmolytic jaundice the test is negative, but uniformly positive results are obtained in conditions that damage and destroy the hepatic parenchyma. Thus cirrhosis, acute or subacute atrophy, catarrhal jaundice, which is perhaps a hepatitis, and neoplasms, all give positive results varying in degree with the extent of the lesiou.

The galactose used must be pure and have a rotatory power of at least 76°. The blood sugar is taken before and every half-hour after the ingestion of the sugar.

The following are two examples—one a normal individual

and the other a case of cirrhosis in a woman, jaundiced, with an enlarged liver and a caput medusæ :—

	Normal. *Per* 100 *c.c.*	*Cirrhosis.* *Per* 100 *c.c.*
Blood sugar before galactose	85 mg.	80 mg.
Half an hour after	106 „	181 „
One hour after	111 „	190 „
One and a half hours after	90 „	140 „
Two hours after	89 „	137 „
Urine	Negative	3 g. of galactose passed.

This test has been used extensively on the Continent, more especially by Bauer, Kahler and Marchoid, and also in America by Rowe (14).

Investigation of the Antitoxic Powers of the Liver

It is well known that certain drugs and organic bodies are combined with detoxicating groups by the liver. Perhaps the best known reaction is that obtained with drugs, such as camphor and salicylates and their derivatives. The administration of these bodies is followed by their excretion in conjugation with glycuronic acid in the urine. In hepatic derangements it is stated that conjugation does not occur and glycuronic acid is absent from the urine.

The Glycuronic Acid, or Roger's Reaction (15).—The patient is given 5 gr. of aspirin, or of salicylate of soda, by the mouth, or 5 gr. of camphor in olive oil are injected subcutaneously, and the following twenty-four hours' specimen of urine is collected and tested for glycuronic acid by Tollen's reaction.

To 20 c.c. of urine, 5 c.c. of 10% basic lead acetate solution are added, and the solution is filtered. Ten c.c. of the clear filtrate are measured off, 5 c.c. of concentrated HCl and 1 c.c. of a 1% solution of naphthoresorcin in alcohol are added. The tube is then heated in a boiling water bath for fifteen minutes, and is removed and cooled. Two c.c. of ether are

added, and the tube is inverted several times. If glycuronates be present the ethereal layer will assume a purple tinge, thus indicating normal hepatic function; if no glycuronates be present an abnormal hepatic function is indicated. A red colour does not constitute a positive reaction.

The Indican Reaction.—The liver is supposed to destroy indican, hence spontaneous indicanuria, or indicanuria after a provocative dose of indol, is said to indicate liver inefficiency. Tests depending upon an increase in the ethereal sulphates after the administration of substances such as thymol have been described, but since these reactions have never been in general use, and have proved unsatisfactory, they will not be discussed.

Investigation of the so-called Hæmopoietic Functions

The Coagulation Time of the Blood.—This is said to be increased in disorders of the liver. The coagulation time may be determined by various methods, such as that of Wright and Colebrook (16), who draw the blood up from a finger puncture into a series of capillary tubes. The tubes are then collected into a bunch and a rubber band is arranged so that the ends are covered. Noting the time, the bundle is dropped into a water bath kept at a constant temperature of 37° C. Tubes are removed at the end of one, two, three to four minutes, and coagulation is observed by attempting to blow out the blood. By this method the coagulation time for normal blood is found to be three and a half minutes.

The Fibrinogen Content of the Blood.—Since the coagulation time of the blood is increased, it would be expected that the fibrinogen content would be decreased in hepatic inefficiency. Hurwitz states that this test is very useful, and recommends the following technique: Twenty c.c. of oxalated plasma are heated to 59° C. for twenty minutes, when the fibrinogen is precipitated. This is centrifuged off, washed in

water, alcohol, ether, and is finally dried and weighed. Alternatively, the nitrogen content of the plasma and serum of the blood in question can be estimated, and the fibrinogen content arrived at by subtraction. Hurwitz (17) states that normally 0·3 to 0·4 g. of fibrinogen per 100 c.c. is present. This may be reduced to 0·05 g. or less in hepatic disorders.

A Colorimetric Method for the Determination of Fibrinogen (18).—*Reagents.*—1. 0·8% NaCl solution.

2. 2·5% $CaCl_2$ solution.

3. 1% NaOH solution.

4. 5% H_2SO_4.

5. Phenol reagent. This reagent is prepared as follows: Boil together for two hours (using a reflux condenser) 100 g. of sodium tungstate, 20 g. phosphomolybdic acid (or an equivalent of molybdic acid), 50 c.c. of phosphoric acid (85 per cent.) and 75 c.c. of distilled water. After the period of heating, cool, dilute to 1 litre with distilled water and filter if necessary.

6. 20% Na_2CO_3 solution.

Standard Solution.—A convenient standard is made by dissolving 50 mg. of tyrosine in 250 c.c. of 0·1 N.HCl.

Method.—Measure 1 c.c. of plasma (from oxalated blood) into a flask containing 28 c.c. of 0·8% NaCl solution, and 1 c.c. of 2·5% $CaCl_2$ solution. Mix and allow to stand undisturbed for twenty minutes. Break up the jelly, shaking slightly, and transfer it to a dry filter. While filtering, a fine glass rod with a pointed end is inserted into the jelly and whirled gently round. All the fibrin will stick to the rod. Slip the fibrin off the rod and press it between dry filter paper, removing as much of the liquid as possible. Transfer it to a 15 c.c. centrifuge tube, add 4 c.c. of a 1% NaOH solution. The tube is now placed in a boiling water bath, and the contents stirred with a glass rod until the fibrin lump has completely dissolved, leaving the calcium oxalate in suspension. Add 10 c.c. of water, mix and centrifuge. The supernatant fluid is transferred to a 25 c.c. volumetric flask, cooled, and 1 c.c. of a 5% H_2SO_4 solution, and 0·5 c.c. of the phenol

reagent added ; dilute to about 20 c.c. with distilled water. Add 3 c.c. of 20% Na_2CO_3 solution, shake, add 1 drop of ether to dispel the foam, make up to volume and mix.

The standard should be prepared at the same time as the unknown. One c.c. of the tyrosine solution is measured out into a 25 c.c. flask, and sulphuric acid, phenol reagent, etc., are added exactly as in the unknown. Leave standing for fifteen minutes before making the colour comparison.

Calculation.—If the standard be set at 20 and the reading of the unknown be R, then the amount of the apparent tyrosine determined is $\frac{20}{R} \times 0{\cdot}2$ mg. Since 1 mg. of tyrosine = 16·4 mg. of fibrin, the amount of fibrin in 1 c.c. of plasma is $\frac{20}{R} \times 0{\cdot}2 \times 16{\cdot}4$ mg., or the percentage of fibrin = $\frac{20}{R} \times 0{\cdot}328$.

Estimation of Blood Lipase (Whipple (19)).—We employ the method of Lowenhart (20). Into four test tubes 1 c.c. of serum is placed, together with 0·3 c.c. of toluene to prevent decomposition ; 3 c.c. of water are then added to each tube, making the total volume 4 c.c. To two of the tubes 0·26 c.c. of ethyl butyrate is added, and all four tubes are incubated for eighteen to twenty-four hours in an incubator at 37° C. At the end of this time the tubes are removed and a drop of azolitmin solution is added to each. The tubes containing serum alone are alkaline, and are titrated with $\frac{N}{10}$ acid. The other tubes, in which the lipase will have produced butyric acid, are acid in reaction, and are consequently titrated with $\frac{N}{10}$ alkali. The amount of lipolytic action is the sum of the amounts of acid and alkali used. Normally this is between 0·2 and 0·3 c.c. ; values above these figures,

indicating increase of lipase, are said to point to derangement of hepatic function.

Widal's Test (21).—**The Hæmoclasic Crisis.**—That a meal is followed by leucocytosis has been known for a very long time. Widal, working on various forms of protein and colloidal shock, demonstrated that this reaction depended upon the functional integrity of the liver. In a person with a deranged hepatic function, a meal is followed either by a leucopenia, or by no rise in the count. This was made the basis of a test, which consists in making a white count before breakfast, and then giving 7 oz. of milk to drink. Counts are then taken at intervals of twenty and forty minutes. Normally there is a leucocytosis up to + 3,000 per c.mm. at the end of twenty minutes, although, according to Wilson (22) this may be delayed until the forty minutes' observation. Where the liver is damaged, however, the leucocyte count remains constant, or else falls. Wilson states that the hæmoclasic reaction is also positive in cases of asthma, epilepsy and certain infections. He also states that it yields positive information at a much earlier stage of liver damage than the other tests, with the possible exception of the lævulose tolerance test. A great deal of work has been done on this reaction, especially in France (Arloing and Langeron (23), Zehnter (24), Feinblatt (25), Erdmann (26)).

Investigation of the Global Capacity of the Liver to eliminate Foreign Substances

The Phenoltetrachlorphthalein Test.—This test was devised by Rowntree, Hurwitz and Bloomfield (27), who noted that this substance, when injected subcutaneously, escapes from the body exclusively *viâ* the biliary passages. Reabsorption from the large gut takes place to a slight extent, but, since the action of the substance itself is purgative, very little absorption occurs. By careful animal work, the originators of this test were able to prove that the

excretion of the drug diminished in proportion to the amount of liver tissue damaged. From these various experiments they evolved the following test :—

2·5 g. of phenoltetrachlorphthalein are weighed into a flask containing 5 c.c. of 2N.NaOH and 45 c.c. of sterile water. This is boiled under a reflux condenser for twenty minutes, filtered, and the volume is made up to 50 c.c. A 5% solution results. This can be procured already made up, but the above authors recommend that it should be freshly prepared every few days. The night before the test the patient is given a purge, and on the following morning 8 c.c. of the solution are injected intravenously. The stools are collected for forty-eight hours after. It is essential that the patient be purged throughout the test. The fæces are then shaken for twenty minutes with 1 to 1·5 litres of water, and one-tenth of the volume is decanted. Five c.c. of 40% NaOH are added, and the mixture turns a dull red colour. After thorough shaking 100 c.c. are decanted into a 200 c.c. measuring flask containing 5 c.c. of saturated basic lead acetate solution. The colour disappears, and a bulky precipitate settles to the bottom. Five c.c. of 40% NaOH are added, thus restoring the colour, and the volume is made up to 200 c.c. The colour should not be deepened by the addition of more NaOH. The solution is allowed to stand for a short while until the supernatant fluid is clear. In the meantime a standard solution of the dye is prepared by taking 0·4 c.c. of the original 5% solution, adding sufficient 40% NaOH to produce a permanent colour, and making the volume up to 1,000 c.c. A small portion of the clear supernatant fluid of the test is removed, and compared in a colorimeter with the standard. By calculation the percentage recovery of the dye can be determined. Any recovery below 30% is regarded as pathological. Chesney, Marshall and Rowntree (28) state that the test yields very satisfactory information in advanced cirrhosis, cancer and syphilis affecting the liver.

Several important papers have been published upon the

phenoltetrachlorphthalein test since Rowntree and his co-workers evolved the method. An admirable summary can be found in a paper by Boardman and Schoonmaker, in 1924 (29). The main advances have consisted in improvements and modification of the original technique. Thus MacNeil (30), in 1916, suggested that the excretion by the liver might be judged by aspirating the duodenal contents through a tube. His method consists of injecting 40 mg. of the dye intravenously into a patient with an Einhorn's duodenal tube *in situ*. Although difficulty was occasionally experienced in the aspiration of specimens and in the detection of the first appearance of the dye in the presence of bile pigment, very satisfactory results were obtained by analysing a two hours' specimen of duodenal contents.

His results can be tabulated as follows :—

Case.	*Time of appearance of dye in duodenal contents.*	*Percentage output of dye at the end of two hours.*
Normal . . .	12–25 minutes ..	10%
Atrophic cirrhosis . .	28–45 ,, ..	10%
Extensive carcinomatosis	28–45 ,, ..	10%

Since the percentage output at the end of two hours is the same in all cases, MacNeil concluded that only the time interval elapsing between the injection and the appearance of the dye in the duodenal contents was of value.

In 1921 Aaron, Beck and Schneider (31) simplified the above technique by modifying the method used in preparation of the dye for injection, and also they stated that difficulties in aspiration could be overcome by giving 500 c.c. of cool water to drink prior to the injection. They employ 50 mg. of phenoltetrachlorphthalein injected intravenously. Their results were exactly similar to those of MacNeil.

A further modification of the phenoltetrachlorphthalein test has been described by Rosenthal (32), in which the excretion of the dye is judged by estimation of its rate of

disappearance from the blood following intravenous injection. The dye is obtained in ampoules, containing a solution of 5 mg. to 0·1 c.c., and 5 mg. of dye are injected intravenously for each kg. of the patient's body weight. The requisite number of ampoules is emptied into 250 to 300 c.c. of normal saline, which are infused into a vein. The needle is washed out into the vein with a few c.c. of saline to prevent local extravasation of the dye. Exactly a quarter of an hour after the injection has been given, the patient is bled with a dry needle into a dry centrifuge tube, and this process is repeated after an hour (from the time of injection). About 5 c.c. of blood are collected at each vein puncture. The blood is allowed to clot, and after separation, the tubes are centrifugated, and the clear serum is pipetted off. Hæmolysis must be avoided at all costs, and this, in our experience, can only be done by leaving the clot to separate of its own accord. If the blood be stirred or interfered with in any way, then hæmolysis is almost bound to occur. The amount of dye present in each specimen of serum is now determined colorimetrically, matching against a standard.

Method.—*Preparation of the Standard.*—0·2 c.c. of the dye solution from the ampoules, which is 5%, and containing 10 mg., is diluted to 100 c.c. with distilled water. The colour developed by rendering this solution alkaline is taken as 100%, and is reckoned as the colour which would have been given by the patient's blood if no dye had been excreted. The following series of dilutions are set up :—

Amount of "100%" standard in c.c.	2·5	2·0	1·5	1·0	0·5	0·2
Amount of distilled water in c.c.	7·5	8·0	8·5	9·0	9·5	9·8
Resulting dilution of standard in percentage . . .	25	20	15	10	5	2

One drop of 5% NaOH is added to each tube. The small test tubes of some type of comparator should be used. That of Cole and Onslow is quite suitable for this test.

The serum is divided between two test tubes of the same

type as those containing the standard. To one add one drop of 5% NaOH, but leave the other untouched, unless the serum be tinted with hæmoglobin, in which case add 2 to 3 drops of hydrochloric acid. The alkaline serum is matched in the comparator against the dilutions of the standard, whilst any colour due to the serum itself is controlled by superimposing the second tube of serum on the standard in the usual manner. The particular dilution of the standard corresponding to the alkaline serum records the percentage of dye remaining unexcreted in the serum.

Findings.—In normal persons, from 2% to 6% of dye remains in the circulation after 15 minutes, whilst at the end of an hour practically none remains. In hepatic disorders, there is an appreciable amount in the circulation at the end of an hour. Rosenthal also noted that the excretory power of the liver did not run parallel with the degree of icterus.

Cholecystography

To Graham (33) first belongs the credit for demonstrating the fact that the opacity to X-rays of some of the substances used for testing the global capacity of the liver could be employed as a clinical means of obtaining cholecystograms. He showed that intravenous injection of the sodium salt of tetrabromphenolphthalein is relatively non-toxic, that this body is excreted almost exclusively by the liver into the bile, and provided that the ducts and gall-bladder are not obstructed the salt accumulates in them, thus rendering X-ray photography possible.

Many derivatives were tested; the calcium salts were found to be toxic and relatively insoluble (34), and further observations by Milliken and Whitaker (35) showed that the sodium salt of the tetraiodophenolphthalein, although no more toxic when in a state of great purity, is more opaque to the X-rays, so that a smaller dose is required. Graham (36) and Carman (37) found that the iodo-salt, when specially purified, is actually less toxic than the brom-preparation.

Intravenous Injection.—Graham's technique is as follows : The patient has his ordinary meals the day before the injection. The injections are given early in the morning, between 7.30 and 9.30. No breakfast is taken, and no lunch except a glass of milk, if the patient be hungry. No protein is given in the evening meal on the day of the injection. Water may be drunk if required, during the day. Five grams of the sodium salt of the tetrabromphenolphthalein are dissolved in 40 c.c. of triply distilled water, and the solution is boiled for 20 to 30 minutes. Twenty c.c. are injected intravenously very slowly, taking 5 to 7 minutes, followed half an hour later by the second 20 c.c. It is advisable to wash through the needle with a little normal saline, so as to prevent extravasation and consequent irritation with the drug. Smaller quantities of the iodo-salt are required, usually about 3 g. for an adult, or 0·048 g. per kg. body weight. This is now usually dissolved in 40 c.c. of triply distilled water and is given in one injection warmed to body temperature. The solution must be freshly prepared and not made up overnight. Adrenalin solution, 1 in 1,000, is kept handy in case of collapse. Occasionally nausea and vomiting, accompanied by a marked fall in blood pressure, even down to 70 mm. Hg. systolic, have been recorded, but the injection of 10 minims of the adrenalin solution restores the patient. If the preliminary injections of the sodium salt have been given very slowly, it is stated that toxic symptoms are very rare. Carman (37), however, reports a series of 178 patients injected intravenously with the brom-salt, and 32 with the iodo-salt. In the former series reactions occurred in 50%, which were severe in 2% of the cases ; in the latter series, however, in which the iodo-salt was used, no reactions were observed. Raison (38), however, is of the opinion that the iodo-salt is so toxic that it should not be administered intravenously. For the forty-eight hours following the injection, 40 gr. of sodium bicarbonate should be taken every three hours, day and night, while the patient

is awake. Radiograms are taken three and six hours after the injection of the dye, and if the gall-bladder shadow is visualised a meal rich in fats, such as scrambled eggs, is then given to stimulate contraction of the gall-bladder. An X-ray is taken two hours later in order to see that the contraction is taking place normally. The healthy gall-bladder should increase in size up to the time of the fatty meal and subsequently diminish. Absence of this alteration in size of the shadow indicates deficient distensibility and contractility of the gall-bladder.

Oral Administration.—In view of the frequency with which toxic results have followed the intravenous administration of the tetrabromphenolphthalein salt, many workers are unwilling to use this route, although with the specially purified iodo-salt, freshly dissolved as mentioned above, reactions are much less likely to occur. If the salt be given by mouth, there is very little, if any, risk of provoking a toxic reaction. Both brom- and iodo-salts have been used, and they can be given in gelatine capsules, coated with keratin.

Carman (37) has reported a series of 800 cases in which he gave the brom-salt by mouth, in doses, varying according to the weight of the patient, of 4 to 7 g. Each capsule contains $\frac{1}{2}$ to 1 g., and they are taken at intervals during the evening meal, a considerable amount of water being consumed. No food is eaten subsequently until all the photographs required have been taken. Slightly smaller doses of the iodo-salt are used. They are put up in capsules containing $\frac{1}{3}$ g. (5 gr.), and the routine observed is as follows : The patient takes a light supper at 7 p.m., containing no fat. At 10 p.m., when in bed, he swallows two capsules and washes them down with water. He takes two more capsules every quarter of an hour, drinking water freely all the time, until ten capsules in all have been swallowed. He then lies on the right side and continues to drink water from time to time until he falls asleep. No aperient must be taken that evening. Nothing is taken next day by mouth until the afternoon. Radio-

grams are made at 10 a.m., 11 a.m., and at 4 p.m. A cup of tea and a little thin bread and butter may then be eaten and another X-ray taken at 5 p.m. to see the effect of the meal upon the shadow. It may also be necessary to take a further radiogram at 10 a.m. the next morning.

Before normal shadows can be obtained the following conditions must prevail :—

1. The drug, if given by mouth, must be absorbed from the alimentary canal.

2. It must be freely excreted by the liver, and, consequently, if there be severe cirrhosis this may be impossible.

3. The hepatic and cystic ducts must be patent, otherwise the gall-bladder will not fill.

4. The gall-bladder and bile ducts must be patent, or the dye will not be able either to enter or to leave the gall-bladder.

5. There must be no fistulous communication between the gall-bladder or other part of the biliary tract and any other viscus, for if so the drug will merely drain away.

According to Carman and Counsellor (39) it is unwise, in view of the possible toxic effects, to perform the test if definite obstruction be known to exist. They also state that it is dangerous in cases of cardiac or vascular disease.

Results of the Test.—1. A normal shadow of the gall-bladder may be obtained, showing first an increased and then a diminished density, and also an increase and diminution in size.

2. The shadow may be mottled. This may be due to a variety of causes. Thus gall-stones may be seen which are more opaque to the rays than is the dye, or cholesterin stones may be present which are less opaque. Gas in the intestine, lying in front of the gall-bladder may also produce a mottled effect, or a papilloma in the gall-bladder may affect the shadow.

3. The contour of the shadow may be deformed owing to inflammatory or other structural changes in the gall-bladder.

4. There may be delay in filling or emptying owing to a sluggishness in the hepatic excretion or to obstructive causes.

5. A negative result may be obtained, no shadow being produced. In the case of intravenous administration this almost invariably implies a pathological condition preventing the dye entering the gall-bladder. With the oral method, however, more uncertainty exists, for there may be failure of absorption of the dye, and the capsules may even be seen by X-rays lying undissolved in the intestine. Provided that the capsules have been correctly taken, have not been vomited, and the dye has been absorbed into the circulation, failure to obtain a shadow usually indicates disease, which is most commonly due to obstruction of the cystic duct, often by a stone.

In the accompanying plate (Fig. 4) a normal cholecystogram is shown, taken fourteen hours after the oral administration of sodium tetraiodophenolphthalein and an abnormal one (Fig. 5), taken twelve hours after intravenous injection of the dye, showing the gall-bladder filled with the dye, four cholesterin stones, and one cholesterin stone with a calcium nucleus.

Investigation of the Duodenal Contents

Lyon (40) has evolved a method for investigating the biliary tract. By the procedure about to be described, it is possible to obtain specimens of bile for chemical and bacteriological examination. The fasting patient swallows a duodenal tube, and, after half an hour, a small quantity of fluid is aspirated. If this be bile-stained and alkaline in reaction it is safe to conclude that the tip of the tube has traversed the pylorus. A syringeful of air is then blown into the duodenum, and a specimen of the contents is withdrawn for bacteriological examination. 50 or 100 c.c. of 25% magnesium sulphate solution are then injected into the duodenum. After five minutes, specimens of bile are withdrawn. The test depends upon the fact that Meltzer (41)

FIG. 4.—Normal cholecystogram.

[*To face p.* 172.

showed that the application of magnesium sulphate to the ampulla of Vater causes the gall-bladder to contract and empty itself. Lyon states that the first sample aspirated after the injection of magnesium sulphate solution should be of a transparent, golden-yellow colour, and is derived from the ducts. The second specimen is darker, more viscid, and consists of gall-bladder bile. The third is thin and watery, being derived from the liver. Bacteriological and cytological examinations of these fractions are said to yield information as to the condition of the various parts of the biliary tract. It is only of use in the diagnosis of inflammatory and infective conditions.

Conclusions.—The description just given contains only the better known tests for hepatic function, and it must be realised that there are literally dozens left unmentioned.

From this it would appear all but impossible to draw any hard and fast conclusions as to the value of any particular test, or to state that such and such a test is always reliable.

At the outset, it is only fair to point out that the response of normal people to various experimental conditions has by no means been carefully or fully worked out, and it seems that a great many of the tests of function should first be tried on much larger groups of normal people.

Taking the tests according to their groups, we have to consider first those depending upon the pigmentary functions of the liver. Tests in this group are used extensively and yield very reliable results. They do not always, however, indicate early changes. MacCormac and one of us (E. C. D.) (42) used Fouchet's test, and the urobilinogen and urobilin reactions for studying the hepatic changes in salvarsan treatment, and similar tests were used with Comyns Berkeley and Walker (43), for the study of liver function in eclampsia. In general hospital work they have been found very useful by most observers. It is difficult to estimate the exact clinical value of the van den Bergh reaction.

Speaking of this test, Langdon Brown (44), quoting Andrewes, states :—

" 1. As a test of liver function it is very gross, being, however, a little more sensitive than clinical evidence of jaundice. Thus several cases of cirrhosis gave normal results.

" 2. As an aid to diagnosis of the cause of jaundice, it is of very little value. A well-marked indirect reaction indicates catarrhal jaundice rather than stone. A direct reaction may mean either.

" 3. In anæmia an indirect reaction above normal indicates a hæmolytic cause, probably pernicious rather than secondary. The test is probably rather more reliable than examination for urobilinuria.

" 4. The estimation of bilirubin in blood-stained fluids (pleural, peritoneal, cerebro-spinal) can decide whether the bleeding is old or produced at the time of puncture.

" 5. With direct reactions above 3·5 units, bile will be present in the urine ; with direct reactions below this figure, and with indirect reactions, bile will be absent there.

" One must conclude, I fear, from this careful study, that in its present form the test is of very limited value. It will be noted that the lævulose test has given more positive evidence than van den Bergh's that catarrhal jaundice is often a hepatitis."

The second group includes the metabolic function tests. It would appear impossible to demonstrate fine hepatic lesions by metabolic changes, since the work of F. C. Mann (45) has shown that four-fifths of an animal's liver can be removed without causing any symptoms other than shock from the operation. If more of the liver be removed the animal has convulsions, as the result of hypoglycæmia, and can be instantly revived by the administration of glucose solutions. This, after all, is only the experimental confirmation of what is frequently seen clinically. A liver may be riddled with secondary deposits of malignant growth, and yet the only symptom of deficiency in its function is jaundice.

Both clinical and experimental evidence point to the remarkable powers of a badly damaged liver to carry on its normal functions, and, at the same time, to the impossibility of demonstrating fine hepatic lesions by means of tests depending on the metabolic functions of the liver. Possible exceptions to this rule would appear to be the lævulose and galactose tolerance tests. The majority of observers are agreed that this method is reliable, and yields valuable information (Spence and Brett (13), Tallerman (46)). The third group of tests has never found extensive use in this country.

We have found that the camphor reaction is quite reliable, and that failure to evoke a response must be regarded as a very grave sign. Roger states that death usually supervenes in a period of one year—a statement with which we are in complete agreement. The blood lipase test in Group IV. has received much attention, yet it seems very inadvisable to place much reliance upon a test in which the technique of the estimation is so rough and admittedly inaccurate.

Widal's hæmoclasic reaction and the phenoltetrachlorphthalein test are held to be quite reliable.

Admittedly it is possible to state with relative surety, on the evidence supplied by these tests, that there is derangement of hepatic function, but, unfortunately, it is impossible to state more. Cirrhosis, carcinoma, fatty infiltration, all produce more or less the same picture, and, since a diagnosis cannot be ventured on the results of hepatic efficiency tests, they must be considered somewhat of a failure from the medical aspect.

REFERENCES

1. Fouchet.
Compt. Rend. Soc. Biol., 1917, lxxx., 826.
2. Ehrlich.
Centralbt. f. klin. Med., 1886, viii., 593.
3. Schlesinger.
Deutsch. med. Woch., 1903, xxix., 561.
4. McNee.
Quart. Journ. Med., 1923, xvi., 390.

5. Eppinger.
Ergeb. der inner. med. v. Kinderheilk, 1908, i., 1107.
6. Aschoff.
Münch. med. Woch., 1922, lxix., 1352.
7. van den Bergh.
"*Der Gallenfarbstoff im Blute*," 1918. Leiden.
8. McNee and Keefer.
Brit. Med. Journ., 1925, ii., 52; also McNee, *ibid.*, 1922, i., 716.
9. Strauss.
Deutsch. med. Woch., 1901, xxvii., 757.
10. Wörner and Reiss.
Deutsch. med. Woch., 1914, xl., 907.
11. Churchman.
Johns Hopkins Hosp. Bull., 1912, xxiii., 10.
12. Shirokauer.
Zeit. f. klin. Med., 1913, lxxviii., 462.
13. Spence and Brett.
Lancet, 1921, ii., 1362.
14. Bauer.
Wiener Archiv. f. inner. Med., 1923, vi., 9.
Kahler and Marchoid.
Wiener klin. Woch., 1922, *Nr.* 18, 414.
Rowe.
Arch. Int. Med., 1924, xxxiv., 388.
15. Roger.
Presse Medicale, 1916, xxiv., 217. See also Gautier.
Ibid., 1916, xxiv., 339.
16. Wright and Colebrook.
"*Technique of the Teat and Capillary Glass Tube*," 1921, 109. Constable, London.
17. Hurwitz.
Journ. Exp. Med., 1911, xiii., 136.
18. Wu.
Journ. Biol. Chem., 1922, li., 33.
19. Whipple.
Johns Hopkins Hosp. Bull., 1913, xxiv., 207.
20. Lowenhart.
Amer. Journ. Physiol., 1902, vi., 331.
21. Widal.
Presse Medicale, 1920, xxviii., 893, and 1921, xxix., 121, Paris.
22. Wilson.
Brit. Med. Journ., 1922, i., 1061.
23. Arloing and Langeron.
Bull. Acad. Med., 1922, lxxxviii., 141.
24. Zehnter.
Paris Méd., 1922, xiv., 281.

25. Feinblatt.
Journ. Amer. Med. Assocn., 1923, lxxx., 613.
26. Erdmann.
Med. klin. Berlin., 1922, xiv.
27. Rowntree, Hurwitz and Bloomfield.
Johns Hopkins Hosp. Bull., 1913, xxiv., 327.
28 Chesney, Marshall and Rowntree.
Journ. Amer. Med. Assocn., 1914, lxiii., 1533.
29. Boardman and Schoonmaker.
Amer. Journ. Med. Sci., 1924, clxviii., 688.
30. MacNeil.
Journ. Lab. and Clin. Med., 1915–16, i., 822.
31. Aaron, Beck and Schneider.
Journ. Amer. Med. Assocn., 1921, lxxvii., 41.
32. Rosenthal.
Journ. Pharmacol. and Exp. Therap., 1924, xxiii., 385.
33. Graham and Cole.
Journ. Amer. Med. Assocn., 1924, lxxxii., 613.
34. Graham, Cole and Copher.
Journ. Amer. Med. Assocn., 1924, lxxxii., 1777, and *Annals of Surg.*, 1924, lxxx., 473.
35. Milliken and Whitaker.
Surg., Gynecol., and Obstet., 1925, xl., 646.
36. Graham, Cole and Copher.
Journ. Amer. Med. Assocn., 1925, lxxxiv., 14.
37. Carman.
Lancet, 1925, ii., 67.
38. Raison.
Brit. Med. Journ., 1926, ii., 542.
39. Carman and Counsellor.
Amer. Journ. Roentgenol. and Radium Therapy, 1924, xii., 403.
40. Lyon.
Journ. Amer. Med. Assocn., 1919, lxxiii., 980.
41. Meltzer.
Amer. Journ. Med. Sci., 1917, cliii., 469.
42. MacCormac and Dodds.
Brit. Med. Journ., 1923, ii., 1200.
43. Berkeley, Dodds and Walker.
Journ. Obstet. and Gynæc. of Brit. Empire, 1924, xxxi., 1.
44. Brown.
Brit. Med. Journ., 1923, i., 461.
45. Mann, Bollman and Magath.
Amer. Journ. Physiol., 1924, lxviii., 115; *ibid.*, 1924, lxix., 371.
46. Tallerman.
Quart. Journ. Med., 1923, xvii., 37.

CHAPTER VI

THE STOMACH

Investigation of the Gastric Function

Apart from clinical study, the following methods are available for the investigation of the stomach :—

Investigation of the response to test meals.

One hour method (Ewald (1)).

Fractional method (Rehfuss (2)).

X-ray examination after an opaque meal.

Test Meals

Before commencing to describe these methods, it is essential to realise the various factors controlling the composition of the gastric contents after the administration of a test meal. These may be summarised as follows :—

The volume and nature of fluid in the stomach at the time of giving the meal.

The volume and nature of the meal.

The rate and amount of gastric and salivary secretion.

The condition of the pylorus, with regard to :—

(*a*) Passage of fluid from stomach to duodenum.

(*b*) Regurgitation of fluid from duodenum to stomach.

The composition of a specimen of gastric contents removed one hour after a test meal must depend on every one of these factors ; more especially does this hold if the free HCl and total acidity are used as criteria, since these are markedly affected by the alkaline regurgitation from the duodenum. As all these factors vary even in normal individuals, it would appear all but impossible to obtain anything like comparable

results with a method employing examination at the end of one hour. Thus, at the time of giving the meal one man's stomach may contain no resting juice whilst another's may have as much as 200 c.c. This volume would produce an added effect upon the response to the test meal, so that, even though it were the same as the first man's response, the composition of the first hour's specimen would be entirely different. Any method, therefore, which aims at giving a true estimate of the gastric function must take into account all these factors.

The two techniques will now be described, and the relative value of their findings discussed.

Ewald's Method (1)

The examination is conducted early in the morning, the patient having fasted from the night before. The test meal, consisting of a pint of tea and a small piece ($\frac{1}{2}$ oz.) of toast, is given at 9 a.m., and at 10 a.m. a gastric tube is passed, and as much fluid as possible is aspirated from the stomach. The volume is measured and the analysis is proceeded with.

Qualitative Analysis

General Appearance of the Meal.—Excessive butyric odour, foul smell, colour, etc., are noted. A foul smelling, black-brown solution is strongly suggestive of carcinoma. The presence of blood, either fresh or in the form of "coffee grounds," should be looked for, but the possibility of these being actual coffee grounds, the result of a meal the night before, should be eliminated. The contents may also be examined microscopically—although the information yielded is seldom of great use. Ewald also laid stress upon the volume of gastric contents recovered. Normally, about 60 c.c. can be aspirated. If the quantity be much greater than this it probably means that the pylorus is not functioning properly

and there is retention. If, on the other hand, only a very small quantity can be removed, there is evidence of a rapid emptying time. It is unwise, however, to put too much stress on this, since other factors, such as the amount of gastric secretion, also vary the volume of the "one hour specimen." The contents are then filtered and the following tests are performed :—

Test for Free HCl in Gastric Contents.—Place 2 drops of Günzberg's reagent in a porcelain dish. Evaporate to dryness on a bath or high over a flame without scorching. Add 2 drops of filtered gastric contents and evaporate to dryness as before. If free HCl be present, a bright pink colour appears. Günzberg's reagent is made up from vanillin 1 g., phloroglucin 2 g., alcohol 30 c.c., and should be freshly prepared.

Tests for Lactic Acid in Gastric Contents.—MacLean (3) recommends the following test for lactic acid in gastric contents : Two test tubes are taken and to one about 3 or 4 c.c. of filtered gastric contents are added ; into the other is poured an equal quantity of tap water ; to each are then added a few drops of the following reagent :—

Ferric chloride	5 g.
Mercuric chloride (saturated solution) .	100 c.c.
HCl (concentrated)	1·5 c.c.

If lactic acid be present a reddish colour appears. The water is used to control the colour of the reagent, and by contrasting this with the colour given by the gastric contents a faint positive may be detected.

MacLean has also modified Hopkins' thiophene test for lactic acid.

Solutions.—1. One hundred and eighty g. of lead acetate are added to 500 c.c. water and the mixture is boiled. Whilst still boiling 110 g. of litharge are added with constant stirring, small quantities at a time, so that each addition dissolves completely. When this is complete, the solution is cooled,

diluted to 1 litre with distilled water and allowed to stand over-night. A precipitate settles out and the supernatant fluid is decanted.

2. Concentrated ammonia, sp. gr. 880.

3. Ammonium sulphate solution, saturated.

4. Copper sulphate solution, saturated.

5. Alcoholic thiophene solution; 20 drops thiophene to 100 c.c. absolute alcohol.

Method.—Five c.c. of filtered gastric contents are boiled and 5 c.c. of the basic lead acetate solution are added. The mixture is brought to the boil and then allowed to cool. Five c.c. of ammonia and 6 drops of saturated ammonium sulphate solution are then added, and the whole is mixed thoroughly by shaking and then filtered. Five c.c. of the colourless, clear fluid are evaporated to a very small volume in an evaporating basin on the water-bath. To the residue are added 5 c.c. of concentrated sulphuric acid and 1 drop of the saturated copper sulphate solution, and the tube is heated in a boiling water bath for two minutes. After cooling under the tap, 3 drops of the alcoholic thiophene solution are added and the tube reheated in a boiling water bath. If sufficient lactic acid be present, a cherry red colour will develop within a few seconds.

MacLean states that this test will give a positive reaction when the concentration of lactic acid is about 0·01%.

Whilst the presence of an excessive quantity of lactic acid in the stomach contents is suggestive of gastric carcinoma, there is not sufficient information as to its presence in non-malignant cases to render the test diagnostic.

Test for Blood in Gastric Contents.—Scrape the solid off the paper used for filtration of contents, and suspend in about 1 inch of water in a test tube, boil, cool under tap. Add about ½ inch of glacial acetic acid and shake. Add about 1 inch of ether and invert several times. If the ethereal layer do not separate, add water without shaking. In each of two test tubes place ½ c.c. of alcoholic guaiacum solution

and 2 c.c. of 3% hydrogen peroxide, and add to one of them the clear ether extract with a pipette.

A blue colour with guaiacum and hydrogen peroxide is given not only by hæmoglobin derivatives, but by oxidising enzymes, by salts of iron and copper, and by iodides; the former are destroyed by boiling, the latter are insoluble in ether. Exactly the same technique can be used for the detection of blood in fæces (see p. 196).

Quantitative Analysis

Before proceeding with details of the analysis, it will be as well to refer to the type and distribution of acids in the gastric contents. The acidity is due to a mixture of hydrochloric and certain organic acids, such as lactic and butyric acids. Since the juice contains proteins, a certain amount of buffering and adsorption of acid will take place. Some of the hydrochloric acid will be in a free, ionised form, whilst some will be adsorbed on to the proteins, with the result that its hydrogen ion concentration will be much lower than that of the free. The conditions usual in gastric contents may be summarised as follows :—

1. Free HCl—ionised, high hydrogen ion concentration.
2. Adsorbed or protein HCl—lower hydrogen ion concentration.
3. Organic acids—lower hydrogen ion concentration.
4. Mineral chlorides—neutral bodies.

The object of the Ewald meal is to estimate each of these. The free HCl is easily estimated by titration with standard alkali to the turning point of Toepfer's reagent (*p*H 4). This will include all the free HCl, which can be calculated as a percentage. If the juice be now treated with standard alkali until a pink colour with phenolphthalein results (*p*H 7·9), the acid under headings 2 and 3 will have been neutralised. The total quantity of alkali added for both titrations will give the sum of the acidity due to Nos. 1, 2 and 3, which

is known as the total acidity. No. 2 as a separate identity has yet to be determined. If we take the neutralised juice of the former experiment and evaporate and incinerate, salts will result, but all the HCl together with the existing chlorides will be retained in the form of chlorides. If these be estimated by Volhard's method, the total chlorine content of the juice can be found. This is usually expressed in terms of HCl. If another quantity of juice be measured out, and, without any previous neutralisation, this be evaporated and incinerated, a mixture of inorganic salts will result. The chloride content will represent No. 4, since all the HCl will have been volatilised off.

With the data now at our disposal, we can calculate the following values :—

1. Free HCl, expressed as HCl %.
2. Total chlorine, expressed as HCl %.
3. Mineral chlorides, expressed as HCl %.
4. Total acidity, expressed as c.c. $\frac{N}{10}$ %.

It can readily be seen that if No. 3 be subtracted from No. 2 the resulting value will represent the free and protein HCl—*i.e.*, the sum of Nos. 1 and 2 of the original classification. This is known as the active HCl. If the free HCl content be subtracted from this figure, the protein HCl can be found.

Into two silica dishes 10 c.c. of filtered contents are pipetted, and a drop of Toepfer's reagent is added to one of them. This reagent is red in the range of hydrogen ion concentration corresponding to free HCl. If there be no change of colour, except for the slight alteration in colour due to the reagent, it may be taken that no free HCl is present. $\frac{N}{10}$ alkali is added from a burette until the colour changes from red to yellow, when the volume of alkali used is noted. This indicates the amount of free HCl present. A drop of phenolphthalein is then added. No colour change results,

since the hydrogen ion concentration is well below its turning point. More alkali is added until a faint pink colour is perceptible. The volume added represents the HCl adsorbed on to the proteins, together with such organic acids as lactic and butyric, the hydrogen ion concentration of which lies between *p*H 3·9 and 7·9. The total volume of alkali added for both neutralisations represents the total acidity. One c.c. or so of alkali is then run in to ensure excess, and the dish, together with that containing the untitrated juice, is placed on a water bath and evaporated to dryness, after which both dishes are heated over a bunsen until the contents become incinerated. This process is continued until an incombustible residue is left. They are then set aside to cool, and the ash is extracted with a few c.c. of water. The specimen which was neutralised by $\frac{N}{10}$ alkali previously to incineration has all the chloride present in the ash as metallic chloride. In the unneutralised specimen, however, the free and adsorbed HCl have been volatilised, and all that remains in the ash is the chloride of the metals present in the original gastric contents. This value is known as the mineral chlorides. Five c.c. of pure nitric acid, 20 c.c. of $\frac{N}{10}$ silver nitrate solution, and 1 c.c. of saturated aqueous iron alum are added to each. An immediate precipitate of silver chloride occurs. The excess of silver nitrate solution is estimated by titration with $\frac{N}{10}$ potassium thiocyanate solution until the appearance of a blood-red colour, due to the formation of ferric thiocyanate, indicates the end point. The amount of the thiocyanate solution run in is then read.

Calculation.—This can be worked out from first principles or according to the following formula :—

Free HCl can be expressed as c.c. of $\frac{N}{10}$ %, *i.e.*, amount of

$\frac{N}{10}$ alkali in c.c. required to neutralise 10 c.c. of juice to Toepfer's reagent, multiplied by 10. This may be converted into actual weight of HCl in grams per cent. by multiplying the above figures by 0·0365.

Total acidity is always expressed as c.c. of $\frac{N}{10}$ % and is given by the sum of the above titration figure, and the amount of alkali run in to neutralise the juice to phenolphthalein. This must be multiplied by 10 to give the percentage.

Total chlorides, expressed as HCl, are given by the following formula (20 — volume of thiocyanate added) × 0·0365. This refers to the neutralised juice.

Mineral chlorides, expressed as HCl, are given by a similar calculation from the observations on the unneutralised juice. Here the free and adsorbed HCl have been volatilised during the incineration, leaving only the mineral chlorides.

Active HCl is given by subtracting the percentage of mineral chlorides from the total chlorides. In addition to the free HCl, this includes the HCl adsorbed to protein in the juice.

The following table shows the usual findings by this method :—

Distribution of Chlorine, expressed as g. of HCl %

	Active.	Mineral.	Total.	Volume of Juice.
1. Normal .	0·15 {0·1 free. 0·05 protein.	0·1	0·25	30–60 c.c.
2. Ulcer .	0·24	0·1	0·34	30–60 c.c.
3. Dyspepsia	0·18	0·1	0·28	50–70 c.c.
4. Carcinoma	0·05	0·2	0·25	40–70 c.c.

Fractional Method (*Rehfuss* (2))

The patient is given a light supper, preferably a glass of milkand a charcoal biscuit, the night before, and the examination is conducted on the following morning before any further food or drink has been taken. The patient is made to swallow a modified Einhorn's tube, such as that of Ryle, which consists of a rubber tube (about catheter No. 8 diameter) free at one end, and terminated at the other by an expanded metal bulb. The type we usually employ is completely covered with rubber and has two fairly large holes bored through the tube just above the bulb. The art of swallowing the tube should be carefully explained to the patient, who should take it in with his lips and be told to breathe freely through the nose. The most useful procedure is for the operator to swallow a tube himself in front of the patient in order to demonstrate the simplicity of the operation. When this is done very few failures will be encountered. After it has been swallowed up to the second mark on the tube indicating the depth of the fundus, the stomach is emptied by means of a Record syringe, aspiration being continued until no more fluid can be removed. The volume is noted, and the specimen is set aside. When the volume is less than 20 c.c. it is highly probable that the total fluid has not been recovered ; washing out with 20 c.c. of tap water will in many cases lead to a more complete evacuation. The injection of a syringeful of air may also assist in the aspiration.

The test meal is made by boiling two tablespoonsful of fine breakfast oatmeal in a quart of water until the total bulk is reduced to 1 pint ; the mixture is then strained through coarse muslin and may be flavoured with salt at will. The resultant meal is more readily aspirated than is the tea and bread meal, and its white colour enables the detection of bile or blood to be made easily ; these advantages outweigh its less palatable character.

One pint of this preparation is given to the patient to

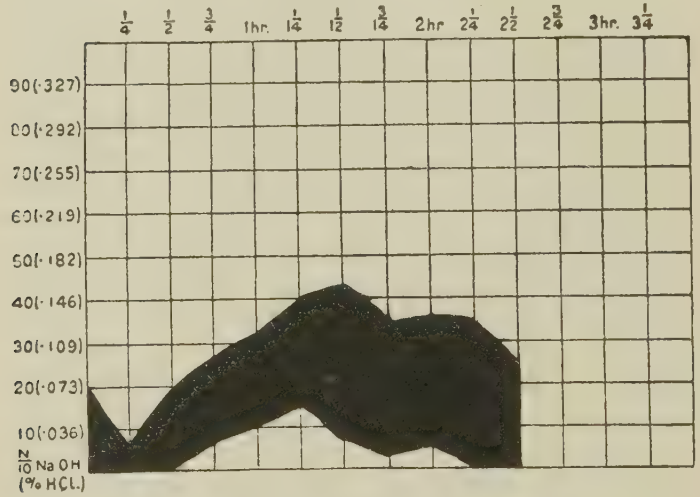
drink as soon as the resting juice has been removed, and 15 c.c. specimens are then withdrawn every fifteen minutes for two and a half hours, or until nothing further can be aspirated. In the former case as much fluid as possible is aspirated and the tube withdrawn. When difficulty is experienced in the aspiration it must be remembered that this may be due to the tube being occluded by solid particles; these may be dispelled by blowing a little air down from the syringe prior to aspiration. A further not infrequent cause of failure to aspirate is the tendency of a strongly-contracting stomach to seize the tube and block its apertures; slight withdrawal or further swallowing of the tube will remedy this.

The specimens are then examined very carefully for blood, bile and mucus. There should be no charcoal in the resting juice if the stomach be normal. Ten c.c. of each specimen, unfiltered, are pipetted off into a series of flasks, Toepfer's reagent and phenolphthalein are added, and the "free" and "total" acidity are estimated by titration with $\frac{N}{10}$ alkali, as described under the previous test. These are expressed as c.c. $\frac{N}{10}$ %, and the results are plotted in the form of a curve similar to the example printed later (Chart I). The Volhard procedure is not usually carried out in this investigation. The inorganic chlorides are therefore not estimated by this method. This is of great importance, for, as Boldyreff (4) first showed in animals and Bolton and Goodhart (5) subsequently demonstrated in man, when the acidity in the stomach reaches a certain level during digestion, the pylorus relaxes and alkaline fluid regurgitates from the duodenum to neutralise it. The acidity, as determined by the titration method, does not therefore represent the amount of HCl secreted, and the curves usually charted as the result of fractional gastric analysis are not in reality secretory curves. The chart and accompanying remarks were kindly

provided for us by T. Izod Bennett (6). The usual emptying time of the gruel meal is from one and a quarter to two and a quarter hours, as determined by tests for starch.

The fall in acidity in the later specimens has been found to be due rather to neutralisation of the acid by alkaline secre-

CHART I



Fractional Gastric Analysis

This shows the area within which fall the readings for free HCl in the majority of normal persons. There is a wide variation amongst normals, but the important fact is that, beginning with a fasting content of low acidity, the first specimen after a meal shows very low HCl, and that there is then a gradual increase in concentration, rarely going above 40, and tending to diminish in later stages. Total acidity is usually some 10 c.c. above the reading for free HCl. The usual emptying time of the gruel meal is from one and a quarter to two and a quarter hours.

tion from the pyloric mucosa and to regurgitation, than to diminution in the secretion (Bolton and Goodhart (5) and Baird, Campbell and Hern (7)). The fall is frequently heralded by the appearance of bile in the contents.

If the total chlorides be estimated by the method given (see p. 183) in each specimen, a continuous rise is found. It is claimed by many workers that this curve represents the

true secretory curve. MacLean and his co-workers (8), however, deny the importance of regurgitation as a factor in the diminution of free acidity in the later specimens. They state that in the isolated Pawlow pouch, a similar falling off in acidity occurs, together with a rise in the total chlorides. This they attribute to an actual secretion of neutral chlorides by the stomach. It must be admitted, however, that all the clinical facts and test meal observations fit in so perfectly with the views of Bolton and Goodhart that it is difficult to reject them in favour of those of MacLean.

The volume of resting juice was found to vary markedly in normal people. Thus Bennett and Ryle (9) found that out of twenty-nine students the volume varied between 10 c.c. and 150 c.c., with an average of 54 c.c. The volume of fluid removed at the end of the examination varies mainly with the efficiency of emptying of the stomach. Thus in normal people little or no fluid can be removed, whilst in cases of pyloric obstruction, 200 c.c. and over may be obtained.

The interpretation of the curves depends upon the following factors:

The Quantity and Character of the Resting Juice.—Does this show signs of stagnation, such as a large volume, foul odour, presence of charcoal, and altered blood? Pus should be looked for, both by naked eye and microscopic examination. The character and composition of this juice is, perhaps, the most important part of the examination.

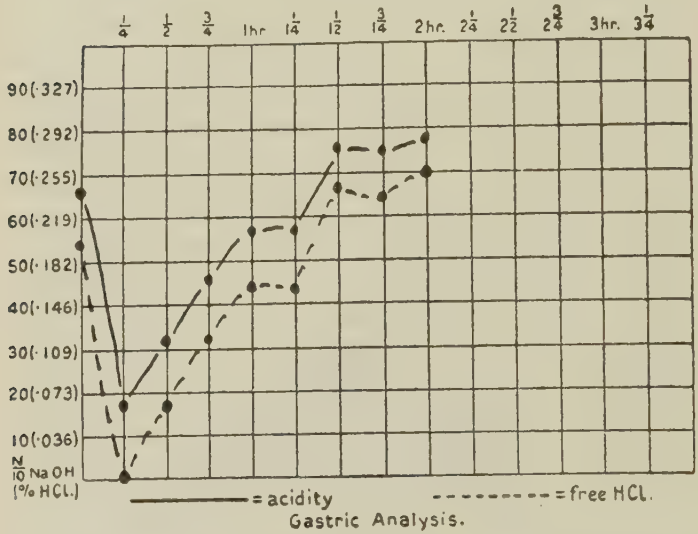
As Izod Bennett (10) has emphasised, it is by the character of the resting juice that carcinoma can be diagnosed.

In this condition the volume is usually high, the free HCl absent or very low. The total acidity is not infrequently from 10 to 25 c.c. $\frac{N}{10}$ % higher than the free HCl, and consists mainly of organic acids, such as lactic and butyric. By far the most important point, however, is the foulness. There

is abundant evidence of stagnation, as shown by the presence of altered blood and charcoal.

The Type of Curve.—The normal curve has been indicated

CHART II



Gastric Analysis.

REPORT

"*Fasting Stomach* contains 30 c.c. of juicé, slightly bile-stained, no food-remnants or charcoal, free HCl in high concentration.

"*Later Specimens. Acidity.*—Free HCl climbs steadily to a concentration considerably above the normal average. Total acid of corresponding height.

"*Bile* absent. *Blood* absent. *Mucus* normal.

"*Motility.*—Starch of meal passes out of stomach in one and a quarter hours, rather rapid.

"*Conclusion.*—The picture is highly suggestive of juxta-pyloric ulcer."

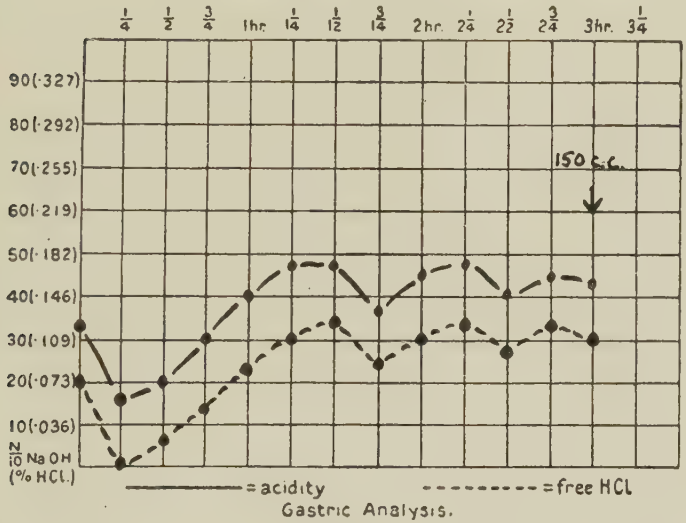
This conclusion was confirmed by operation.

in Chart I, but it must be realised that normal stomachs give widely varying curves, both from the point of view of emptying time and height of secretion. A series of curves typical of the various gastric disorders commonly encountered is included. These were supplied by T. Izod

Bennett (6), and his comments upon them are included. (Charts II, III, IV, V and VI.)

Conclusions.—Firstly, with regard to the Ewald meal, it

CHART III



REPORT

"*Fasting Stomach* contained 70 c.c. of fluid, trace of bile, charcoal and bismuth from previous day, moderate free HCl, total acid rather high.

"*Later Specimens. Acidity.*—Free HCl climbs to a continued plateau of moderate concentration, total acid proportionally rather high.

"*Bile* present in traces in later specimens.

"*Blood* absent. *Mucus* normal.

"*Motility.*—As much as 170 c.c., containing much gruel, were present three and a quarter hours after the meal.

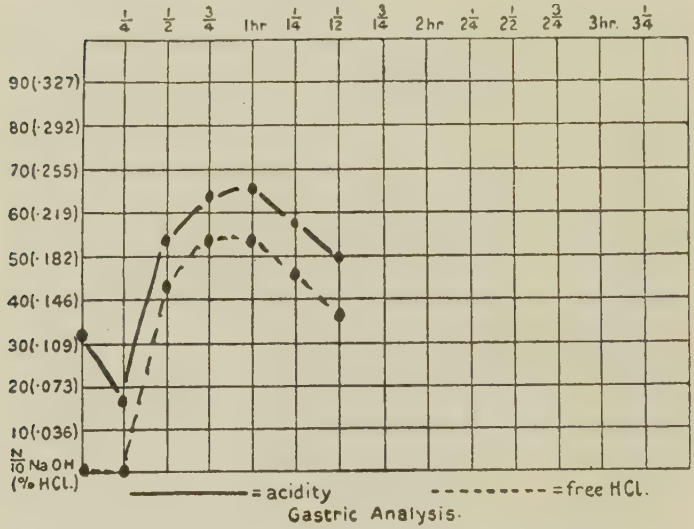
"*Conclusion.*—There is obvious pyloric obstruction, the presence of increasing concentration of free HCl and absence of blood point to this being due to a cicatrising gastric ulcer."

This conclusion was confirmed by operation.

becomes obvious that it is impossible to judge the condition of the stomach from a single hour examination. The reasons for this statement are found in the introductory remarks. If only one specimen can be collected, it is far better to examine the resting juice. This method is employed

extensively in France. From a qualitative and quantitative examination, evidence of retention, hypersecretion and bleeding can be obtained. An examination of the resting juice is of the greatest importance in the diagnosis of gastric

CHART IV



REPORT

"*Fasting Stomach* contains 40 c.c. of fluid, much mucus, charcoal from previous night, altered blood. Foul-smelling. No free HCl, total acid relatively high.

"*Later Specimens. Acidity.*—Free HCl and total acid rather above average concentration.

"*Bile* absent. *Mucus* normal. *Blood* abundant in last specimen.

"*Motility.*—Meal leaves stomach rather rapidly.

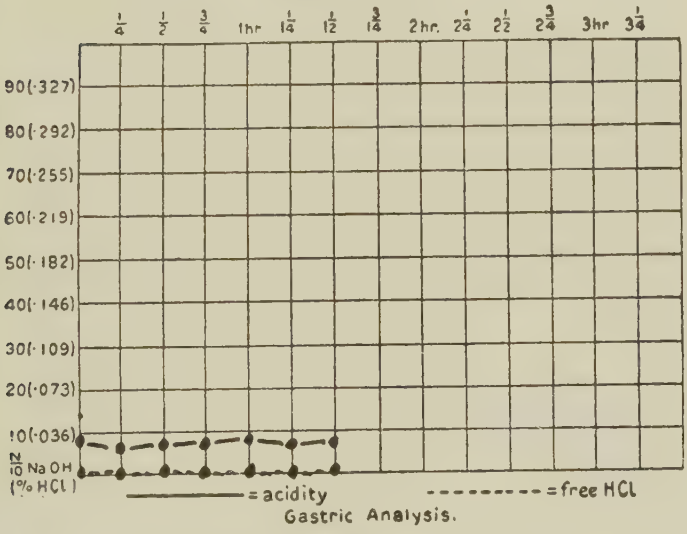
"*Conclusion.*—The character of the fasting stomach content is diagnostic of carcinoma. Later specimens show little abnormality."

Operation confirmed this; this patient was only twenty-two years old.

carcinoma. At the time of its introduction, the fractional method was regarded as being ideal, and as overcoming all the fallacies and difficulties of the earlier methods. Experience has proved, however, that it is far from infallible. Thus Butcher (11) has shown that difficulty in swallowing the

tube in a normal person will cause marked hypoacidity and gastric inertia, and a fallacious curve is thereby obtained. Duthie (12), using a double tube, whereby specimens could be simultaneously obtained from the pyloric and cardiac

CHART V



REPORT

"*Fasting Stomach* contains a few c.c. only of juice, trace of bile, no free HCl, total acid very low.

"*Later Specimens. Acidity.*—HCl completely absent, total acidity also very low.

"*Bile* in last specimens. *Mucus* absent. *Blood* absent.

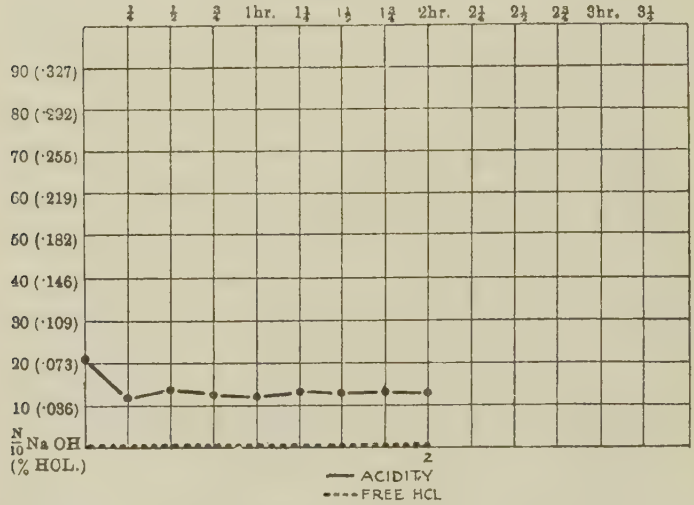
"*Motility.*—Meal has disappeared in three-quarters of an hour, juice extracted with difficulty afterwards.

"*Conclusion.*—Complete achlorhydria, a typical picture of benign Achylia Gastrica."

portions of the stomach, found differences in the acidity of the two specimens, indicating that the position of the tube in the stomach influences the curve obtained in a fractional test meal. Alterations of the normal curves by pathological factors consist mainly in modifying the physiological ones.

Thus there is in reality no new factor added, the disease merely modifying and distorting the normal series of events. There is no evidence that gastric ulceration is inevitably attended by hypersection. The typical climbing curve is best explained on the ground that an ulcer in the region of the pylorus produces spasm, with the result that the meal is

CHART VI



Gastric Analysis.

REPORT

"*Fasting Stomach* contains 40 c.c. of foul dark-coloured juice. This, together with the absence of free HCl and the relatively high total acidity, indicates carcinoma."

This diagnosis was subsequently confirmed at operation.

held longer in the stomach and alkaline regurgitation is absent. Further, the lengthy contact of food with the gastric mucosa prolongs the stimulation for the secretion of acid. This is borne out by the fact that this type of curve is usually given in cases of juxta-pyloric ulcer, and very seldom in ulcers high up the lesser curvature. From this it can be seen that the ulcer does not produce the climbing curve merely

because it is an ulcer, but because it produces pylorospasm. The presence of partially decomposed blood strongly indicates an organic lesion. Bright red blood is usually traumatic, and the possibility of bleeding from spongy gums must always be excluded.

The factors tending to influence the concentration of HCl in the stomach are the following :—

(*a*) Those favouring an increase in the HCl concentration : gastric and duodenal ulcer, gastric crisis of tabes, the " hypersthenic gastric diathesis " and reflex causes, such as cholecystitis and appendicitis, gastric hypersecretion (Reichmann's disease), and chlorosis.

(*b*) Those favouring a decrease in the HCl concentration : gastric carcinoma, pernicious and severe secondary anæmias, and atonic dyspepsia.

The main utility of the fractional test meal may be summarised as follows :—

Examination of the pyloric function.

Diagnosis of juxta-pyloric ulcers and obstruction.

Diagnosis of carcinoma, and investigation of cases of achlorhydria.

The possibility of variation in the curve of an individual from time to time has been urged as a criticism against this method. Bennett and Ryle (9) state that in the re-examination of some of their cases the curve appeared to be remarkably constant, although they were aware that this was not always the case. Lion, Bartle and Ellison (13) state that great variations in the type of curve may be observed in the same individual examined at various times under the same conditions. It seems very doubtful, however, whether these alterations are of the type that would definitely simulate pathological curves, such as those shown in the charts.

In duodenal ulcer, very little may be shown on the curve, unless the case be at the " hurry " stage, when a high concentration of acid, together with a rapid emptying time, will be shown. The fractional test meal is essentially a clinical

test and should be done at the bedside by an experienced person. It is not going too far to state that almost as much can be learnt from the manner in which the specimens are withdrawn, and their naked-eye appearance, as from the quantitative analysis.

The Detection of Blood in Fæces

The importance of testing the fæces for blood should never be lost sight of. Provided that the patient has been on a meat and chlorophyl-free diet for three days prior to the test, a positive reaction, taken in conjunction with clinical and test meal findings, points strongly to an organic lesion of the stomach or duodenum. If a negative result be obtained, a series of further tests should be performed before the absence of blood can be definitely determined.

Benzidine Reaction.—A thin fæcal suspension is made, using about 5 c.c. of distilled water. It is boiled to inactivate oxidising enzymes. To 2 c.c. of a saturated solution of benzidine in glacial acetic acid 3 c.c. of 3% hydrogen peroxide and 2 or 3 drops of the cooled fæcal suspension are added. A clear blue colour develops within one or two minutes in the presence of blood. If the mixture be not shaken a blue ring will form at the top. The latter is suitable for the detection of small traces of blood.

Guaiacum Test.—This has already been described on p. 182.

The tests described will only give positive results if the hæmoglobin has not been broken down beyond hæmatin in the alimentary canal. It is maintained by some that if the bleeding be high up in the alimentary tract, and be slight, decomposition beyond hæmatin may occur, with the result that iron-free compounds, such as porphyrins and hæmochromogen, may be produced, and these would not react with guaiacum or benzidine. Snapper (14) has reviewed this aspect of the subject and states that 25% of gastric and

duodenal ulcer cases show no blood in the fæces, whilst 40% of patients, even on a meat and vegetable-free diet, and with no gastro-intestinal lesions, show traces of occult blood. In malignant disease of the stomach and intestines, blood is always found, provided a search is made for porphyrins, because, in 16% of these cases, all the hæmatin is broken down to these bodies. This breaking down is very common in malignant disease, and if no porphyrins be found in the fæces, this is strongly against a diagnosis of carcinoma of the stomach or intestines. Snapper recommends the following technique :—

One gram of fæces is ground up in a mortar with an excess of acetone, and, after thorough mixing, it is filtered and the solid matter thoroughly washed with more acetone. The solid matter is scraped off the paper and is pressed between fresh paper until it is dried. The granular matter is then ground up in a clean mortar with a small quantity of a solution composed of one part of acetic acid and three parts of acetic ether. As small a quantity of solution as possible is used in order to keep the extract concentrated. On filtration, to a portion of this extract a quarter of its volume of pyridine and two drops of ammonium sulphide are added. The solution is examined with a spectroscope, preferably in a dark room. The colour of the solution prior to adding the ammonium sulphide, which must be fresh, is yellow, and afterwards it turns pink, due to the formation of hæmochromogen. The characteristic band of this substance (in the green, λ 560) may be seen if the specimen be examined at once, but it fades rapidly, and also if there be but little present, it may not be detected. A further 5 c.c. of the extract from the fæces is acidified with 2 c.c. of 5% hydrochloric acid, and 5 c.c. of ether are added. If porphyrin be present, a spectrum with two absorption bands is seen, one in the green (λ 570 to λ 555) and a narrow band in the yellow (λ 605 to λ 600).

X-ray Examination

Examination of the stomach and of the duodenum by means of the X-rays after the ingestion of an *opaque meal* is a useful adjunct in the differential diagnosis of ulcers of these organs.

Investigations should be made both under the screen and with photographs. The opaque meal used in the screen examination usually consists of 3 oz. of barium sulphate in half a pint of milk. By this means the shape of the stomach and its filling properties can be investigated.

The motor power and rate of stomach emptying are better studied with a more solid meal, such as porridge, or bread and milk containing barium sulphate. There should be no residue of such a meal in the stomach after six hours.

Persistent deformity in the outline of the stomach or duodenum is the most reliable evidence in the X-ray diagnosis of ulcer. Thus in gastric ulcer the barium may be seen filling a niche in the wall of the stomach, or there may be a definite organic hour-glass appearance. In the duodenum a persistent alteration in the shape of the duodenal cap may be seen. The X-ray examination will also reveal pyloric obstruction.

Variations in tone or in the position of the stomach are not diagnostic of ulceration. Subsequent examination after the administration of belladonna is of value in distinguishing between constriction of the stomach due to spasm and that due to cicatrisation.

Principles of Treatment of Gastric and Duodenal Ulcers

Various modifications in the treatment of ulcers of the stomach and duodenum have been introduced during recent

years. From the practical point of view the subject can be considered under the following headings :—

The treatment of hæmatemesis due to gastric or duodenal ulceration.

The medical treatment of ulcers not at the time complicated by hæmorrhage.

The indications for surgical treatment of gastric and duodenal ulcers.

Hæmatemesis.—The immediate treatment of acute hæmatemesis due to gastric or duodenal ulceration is almost invariably medical. This has been emphasised at a discussion at the Royal Society of Medicine, at which Paterson (15) pointed out that the probable mortality figures are as follows : Not more than one in nine cases treated medically will die, whereas one in three cases treated by immediate operation will probably die. The surgeon should not, therefore, be called in as a routine measure in such a case, but, despite these figures, there seems little doubt that there are some cases, such as those in which a thickened artery is opened at the base of an ulcer, in which bleeding can only be checked by surgical means.

Medical treatment consists in : (*a*) The patient is put to bed and kept at absolute rest ; (*b*) The immediate injection of $\frac{1}{4}$ gr. morphine in an adult. Willcox (16) has uttered a word of warning in this respect. If there be any doubt as to the nature of the hæmorrhage it is better to withhold the morphine, or, at any rate, to give it in minute doses, for in cases of cirrhosis of the liver, in which the detoxicating power of the liver may be diminished, $\frac{1}{4}$ gr. of morphine may be sufficient to cause death. The morphine assists to control the hæmorrhage by diminishing the peristaltic movements of the stomach ; (*c*) No food must be given by the mouth for at least two to four days. The toilet of the mouth must be carefully attended to, and the mouth may be rinsed out from time to time with water. Ice should not be given to the patient to suck. The bowels should be opened by an enema,

and rectal salines containing 2% glucose given in amounts of 6 to 8 oz. every four hours; (*d*) An intramuscular injection of 1 gr. calcium chloride dissolved in 100 m. of distilled water should be given into the buttock (Willcox) to increase the coagulability of the blood; (*e*) The question of blood transfusion is somewhat debatable. Unless there is great collapse from loss of blood it is better avoided, as it tends to increase the circulation through the bleeding area. However, in very severe cases it may save life.

Other methods which have been recommended, but which are of more doubtful value, include the application of an ice bag to the epigastrium, the subcutaneous injection of 40 c.c. of 10% solution of sterile gelatine daily, and the rectal injection of hot water at 120° to 130° F. Gastric lavage with warm water, if the hæmorrhage and vomiting persist, is a procedure which may be accompanied by risks and which we cannot recommend.

After periods of two to four days, when the bleeding has been arrested, as judged by absence of hæmatemesis and by the general condition of the patient, alkalies should be administered, together with drugs directed to the diminution of gastric secretion. At the same time small feeds of milk (3 to 5 oz.), which may be peptonised, containing 2 dr. of emulsio mag. oxid. to 4 oz. of milk should be given every four hours. Tincture of belladonna (m. x—xv) and olive oil ($\frac{1}{2}$ oz.) should be given alternately before the feeds.

The medical treatment after the hæmorrhage stops is as for the treatment of ulcer not complicated by hæmorrhage, which will now be considered, but in every case that has recovered from a hæmatemesis the advisability of subsequent surgical interference must be determined. This will be referred to under the third heading.

An alternative form of treatment is to start the patient on the Lenhartz diet, as detailed below, while the bleeding is still occurring. This appears physiologically to be an unsound procedure.

The Medical Treatment of Ulcers not Complicated by Hæmorrhage.—The two chief methods now used are :—

1. The Lenhartz treatment (modified).
2. The Sippy treatment (modified).

The Lenhartz Treatment (modified).—Lenhartz (17) introduced his treatment in 1904, with the idea of rapidly giving the patient a diet of adequate caloric value, which will enable him the better to combat the anæmia and debility consequent upon a hæmorrhage. The diet is rich in protein, which should combine with the HCl of the gastric secretion. There is no preliminary period of starvation, and the stomach is not distended with large quantities of food. Small feeds are given frequently and the food is all iced. On the first day the caloric value of the diet is about 100, and at the end of a fortnight it has been raised to 2,200.

The dietary, as originally described by Lenhartz, has been modified by Lambert (18) to suit the American palate, the raw ham being omitted and substituted by an equal amount of cooked chopped chicken, and after the eleventh day various cereals are given instead of rice, and chopped beefsteak and chicken may be taken instead of raw beef.

At St. Bartholomew's Hospital a still further modification is used by Langdon Brown (19). Plasmon is substituted for the meat, and the number of eggs is materially reduced during the second week, whereas pounded fish is added. The dietary is given in the table on p. 202.

The details of its administration are as follows :—

The fresh milk and the raw eggs, beaten up whole, are iced. The eggs and milk are kept in covered glass tumblers surrounded by ice. The spoon is iced. The sugar which is added on the third day is given half in the eggs and milk, and half in the Glaxo. The Glaxo is given in alternate feeds. The patient must not be allowed to feed himself for a fortnight and must eat slowly and masticate well. He is fed with a spoon, the feeds are gradually increased in size up to 5 oz., starting with 1 oz. A feed is given every two hours

Modified Lenhartz Dietary (Langdon Brown).

Day.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Eggs	1	1½	2	2½	3	3½	4	4	3	3	3	2	2	2
Milk (oz.)	3	4½	6	7½	9	10½	12	17	18	19	20	21	22	23
Total bulk of egg and milk together (oz.)	5	7½	10	12½	15	17½	20	25	25	25	25	25	25	25
Glaxo, ½ strength (oz.)	5	7½	10	12½	15	17½	20	25	25	25	25	25	25	25
Sugar, half in Glaxo (dr.)	—	—	6	6	8	8	12	12	14	14	14	14	14	14
Plasmon (dr.)	—	—	—	—	—	2	3	3	3	3	3	3	3	3
Blancmange (oz.)	—	—	—	—	—	—	—	3½	3½	7	7	10	10	10
Rusks (oz.)	—	—	—	—	—	—	—	—	¾	1½	1½	2½	3	4
Pounded fish (oz.)	—	—	—	—	—	—	—	—	2	2	2	2	2	2
Butter (oz.)	—	—	—	—	—	—	—	—	—	¾	1½	1½	1½	1½
Quantity of each fluid feed (oz.)	1	1½	2	2½	3	3½	4	5	5	5	5	5	5	5
Approximate calorie value	106	240	400	475	580	685	825	1,115	1,185	1,650	1,820	2,010	2,080	2,200

from 7 a.m. to 9 p.m. and one at 1 a.m. and at 5 a.m., making ten feeds in the twenty-four hours. An enema should be given every alternate day after the first week if necessary, and if the patient complain of thirst half a pint of warm saline per rectum may be given twice a day, or 1 oz. of water by mouth occasionally after the second day.

The mouth should be washed out before and after feeds with sodium bicarbonate solution (gr. 60 to 5 oz.).

If there be bleeding, bismuth subnitrate may be given, and during the second week iron can be administered by the mouth.

Personal experience has taught us that this form of treatment yields good results and is also appreciated by the patient. The chief objection is that the excess of protein may increase the gastric secretion. Having completed a course of Lenhartz treatment, provided he be free from pain, the patient may be placed on the diet of week 5, of our ulcer dietary (healing stage) described on p. 205. If there be still pain it is wise to adopt the alkali treatment, described below, starting from the beginning.

The Sippy Treatment (modified) or The Alkali Treatment.—Sippy (20) of Chicago, introduced this treatment in 1915. Its object is to protect the ulcer from hydrochloric acid. This is accomplished by giving large doses of alkalies after, and of belladonna before the feeds, and by using as the basis of the diet milk and cream.

The original Sippy treatment, with hourly feeds, and medicines immediately before, and half an hour after the feeds, proved so irksome to the patient and required such attention from the nursing staff and doctors that it was rarely practicable.

Modifications of the Sippy treatment have been found to yield good results and are more easily carried out. The feeds are given at two or two and a half hourly intervals, an alkaline powder is taken immediately after, and olive oil or belladonna before the feeds. There is some difference of opinion as to the best alkalies for the purpose. Sippy (20)

recommended sodium bicarbonate, heavy magnesia and bismuth carbonate. All carbonates have the disadvantage of liberating CO_2 and causing gaseous distention, which proves distressing in some cases. Sodium bicarbonate and magnesium oxide provoke a secondary secretion of gastric juice. Bismuth carbonate has no neutralising power and theoretically is of little value, as it is improbable that it forms a protective coat to the ulcer. Clinically, however, bismuth undoubtedly exerts a sedative effect. Magnesium oxide and magnesium hydroxide are both powerful neutralising agents. Sodium and potassium citrate, tribasic calcium phosphate and tribasic magnesium phosphate neutralise hydrochloric acid without producing an alkaline solution (21), (22).

There is some risk of producing alkalosis if large doses of magnesia or sodium bicarbonate be given, but this risk is probably diminished if citrates or phosphates be employed. The powders which we use are (1) magnesium hydroxide, prepared chalk and bismuth oxycarbonate in equal parts, and (2) tribasic calcium phosphate and tribasic magnesium phosphate in equal parts (Hurst (22)). The amount of magnesium, calcium and bismuth salts can be varied according to whether a laxative or astringent effect is necessary to secure an efficient action of the bowels.

Milk is the best neutralising food substance, and the addition of sodium citrate, gr. 3 to the ounce, not only prevents curds forming, but also increases the neutralising effect. Fatty substances tend to diminish gastric secretion, carbohydrates have little neutralising power.

During the stage of healing of the ulcer the patient must be kept in bed and feeds given at not more than two to two and a half hourly intervals during the day ; by night a feed should only be given if the patient wake. The time for an ulcer to heal, as judged by X-ray appearances and negative tests for occult blood in the fæces, varies according to its size from four to eight weeks. It is improbable that a

deeply excavated and adherent ulcer could heal so quickly. Although some authorities maintain that it is irrational to increase the diet until the ulcer is healed, in our experience this can be done with advantage by carefully graduated changes.

The treatment we adopt is as follows :—

Healing Stage (Diet 1).—*Weeks* 1 *and* 2.—Feeds of 5 oz. are given at 7 a.m., 9 a.m., 11 a.m., 1 p.m., 3 p.m., 5 p.m., 7 p.m., 9 p.m., 11 p.m., and once during the night if awake. The feeds consist of milk (warm and containing 3 gr. sodium citrate to the ounce), Horlick's milk, Benger's food or Glaxovo. At least five of the feeds should be of milk which may be coloured with tea.

Week 3.—Add two raw eggs given in milk, a little thin bread and butter and cream 1 oz. three times a day. In addition, the patient may be given once a day a little of one of the following : sweet jelly, milk jelly, custard or junket. The feeds are still given two-hourly.

Week 4.—The feeds are given every two and a half hours, from 7 a.m. to 10 p.m. They consist of 5 oz. of citrated milk every other feed, the alternate feeds being composed of a similar quantity of potato soup, arrrowroot, cornflour, or milk pudding (sago or tapioca).

Week 5.—The feeds are given every two and a half hours. The milk may now be reduced to three times a day, at 7 a.m., 5 p.m. and 10 p.m. Additions to the diet are pounded fish 2 oz., lightly boiled egg, crisp toast or rusk (well chewed) with butter.

Weeks 6 *to* 8.—Feeds still every two and a half hours. Add 2 to 4 oz. pounded fish or minced meat daily.

Take immediately before three feeds, $\frac{1}{2}$ oz. of olive oil, and immediately before three other feeds, tinct. bellad. m. 5–10 and Aq. chlorof. $\overline{3}\frac{1}{2}$, or Atropin sulphate gr. $\frac{1}{200}$ in $\overline{3}$1 of water. Take one teaspoonful of the alkaline powder after five feeds during the day and two teaspoonfuls the last thing at night, in a little water, during the first two weeks of the treatment.

During the third and fourth weeks take a teaspoonful of the powder three times a day after feeds and two teaspoonfuls at night. In the fifth to eighth weeks take a teaspoonful of the powder twice a day after feeds and two teaspoonfuls at night. The mouth should be cleaned after each feed with bicarbonate of soda solution, gr. 60 to ℥5 of water. No smoking is allowed.

Convalescent Stage (Diet 2).—During this period of about a month the patient is allowed up for gradually increasing periods, and additions are made to the dietary.

7.30 *a.m.*, *Breakfast.*—One egg (lightly boiled), thin bread and butter or crisp toast and butter (to be well chewed), honey or apple jelly, sugar and milky tea.

10 *a.m.*—Milk 5 oz. (containing 15 gr. sodium citrate) or Horlick's milk or Benger's food.

12.30 *p.m.*, *Lunch.*—Milk soup (potato or artichoke), fresh white fish (boiled or steamed), or rabbit, chicken or tender mutton, mashed potato. Custard or milk pudding or stewed apples (with no pips, skin or core), and the juice of an orange.

3 *p.m.*—Thin bread and butter or biscuits and butter and milky tea.

5.30 *p.m.*—Milk 5 oz. (containing 15 gr. sodium citrate) or Horlick's milk or Glaxovo.

8 *p.m.*, *Dinner.*—As for lunch.

10.30 *p.m.*—Milk 5 oz. (containing 15 gr. sodium citrate) or Benger's food.

The alkaline powder should be taken, one teaspoonful after breakfast and two teaspoonfuls last thing at night, and the olive oil or belladonna before breakfast, lunch and dinner. The meals should be small and eaten slowly.

After Treatment (Diet 3).—8 *a.m.*, *Breakfast.*—A selection from porridge, made from very fine oatmeal; egg, boiled, poached or scrambled; toast, well chewed and crisply made; breakfast biscuits; honey; apple jelly; butter; sugar; cream; and milky tea or coffee.

11 *a.m.*—A tumbler of milk, Ovaltine, Horlick's milk, Glaxovo, egg and milk, or Benger's food and a biscuit.

1 *p.m.*, *Lunch.*—A selection from soup, made from milk and vegetables, as potato or artichoke. Fresh white fish, boiled or steamed with white sauce or fish soufflé. Meat, as minced fresh beef, mutton or veal, grilled lamb cutlet, boiled or roast chicken. Vegetables, as mashed potato, steamed or boiled green vegetables, passed through a seive and served with butter. Sweets, as custard, boiled or baked, junket, milk pudding very well cooked, cornflour, stewed apples, with no pips, core or skin, chocolate soufflé. Crisp toast, the juice of one or more oranges, cheese soufflé.

4 *p.m.*—A cup of milky tea, thin bread and butter.

7 *p.m.*, *Dinner.*—As at lunch.

10 *p.m.*—A feed as at 11 a.m.

The breakfast, lunch and dinner should be small meals, well chewed and eaten slowly. The patient should rest for half an hour after meals. No condiments should be eaten, and nothing taken which cannot be reduced to a soft pulp in the mouth. If it be impossible to obtain the liquid feeds between all the meals, a biscuit or some chocolate should be eaten. A teaspoonful of the alkaline powder should be taken every night on retiring to bed.

The Danger of Alkalosis.—Large doses of alkalies, especially of sodium bicarbonate, magnesium oxide and carbonate, may produce toxic symptoms (Hardt and Rivers (23)). This is liable to occur in cases of gastric stasis especially associated with duodenal ulcer or renal insufficiency. It would appear that this observation is difficult to reconcile with the rationale of the alkali treatment of nephritis, for renal insufficiency usually tends to produce acidosis. The symptoms are headache, vertigo, loss of appetite, distaste for milk, malaise, nausea, vomiting, drowsiness or coma. A determination of the alkali reserve of the blood is of value in confirming the diagnosis. In the early stages the symptoms are usually relieved as soon as the alkali is stopped. Hardy (24)

reports a severe case, in which the headache started the day after the treatment commenced, and on the fourth day the patient was desperately ill. The urine contained albumin and granular casts, the blood pressure and blood urea were raised. In such a case all alkalies should be stopped, and glucose given by mouth or in rectal salines, and an intravenous injection made of collosol calcium (1 c.c.), repeated next day if necessary.

Whatever form of treatment be adopted, it is important that, as soon as the patient is in a fit state, a search should be made for any possible focus of infection, which should be eradicated when found. This is a proceeding similar to that which has to be adopted in the treatment of diabetes mellitus. The teeth should be X-rayed, the nose and sinuses examined, and pyorrhœa should be treated. A search for signs of appendicitis or infection of the gall-bladder or urino-genital tract should also be made.

The Medical Treatment of Chronic Ulcers.—This is in all essentials similar to that detailed above for acute ulceration of the stomach or duodenum ; in fact, apart from the history of the duration of the case, it is difficult to distinguish between the two conditions.

Young (25) recommends treatment by feeding through a duodenal tube. This is kept *in situ* for about eighteen days and the patient is fed through it. At the same time alkalies and bismuth may be given by the mouth to neutralise the stomach contents. Smoking appears to have a deleterious effect and should be discontinued.

The Indications for Surgical Treatment.—While, on the one hand, it is clear that in certain circumstances medical measures are of no avail in the treatment of gastric and duodenal ulcers, yet patients are frequently seen who have had operations for these conditions and who have received no permanent benefit. Indeed, their last state is not infrequently worse than their first. It is, therefore, profitable to endeavour to form an opinion as to what constitute the

indications for surgical treatment. In this way patients should derive the maximum benefit possible, and not be subjected to operations which either give no relief or are followed by only a temporary amelioration of the symptoms.

The following are the chief indications for operation :—

Surgical treatment is imperative in cases of perforation or of perigastric abscess.

Surgical treatment should be advised in cases of recurrent hæmorrhage which is not controlled by strict medical treatment.

Surgical treatment may be required in primary hæmorrhage in elderly people, whose vessels are sclerosed and blood pressure raised, and in whom the hæmorrhage is not checked by medical treatment. It is rarely, if ever, required in primary hæmorrhage under other circumstances.

Ulcers associated with organic pyloric obstruction should be operated upon, a gastro-enterostomy being performed. Such cases will not improve with medical treatment.

Recurrent gastric ulcers may require operation.

Organic hour-glass constriction, causing a constantly recurring delay in emptying of the proximal segment, is an indication for operative treatment.

It will be noticed that throughout this section gastric and duodenal ulcers have been considered together, as if their treatment were the same. Duodenal ulcers are of much more frequent occurrence, and are more easy to diagnose. The alkali treatment is unquestionably beneficial for duodenal ulcers ; whether it is so for gastric ulcers is a matter of some doubt.

REFERENCES

1. Ewald.
Klin. der Verdauungs Krankenheiten, 1890, i., 87.
2. Rehfuss.
Amer. Journ. Med. Sci., 1914, cxlvii., 848.
3. MacLean.
"*Modern Views on Digestion and Gastric Disease*," 2nd edn., 1928, 124. Constable & Co., London.

4. Boldyreff.
Quart. Journ. Exp. Physiol., 1914, viii., 1.
5. Bolton and Goodhart.
Lancet, 1922, i., 420.
6. Bennett.
Journ. Clin. Res., 1923, ix.
7. Baird, Campbell and Hern.
Guy's Hosp. Rep., 1924, lxxiv., 37.
8. MacLean and Griffiths.
Journ. Physiol., 1928, lxv., 63.
MacLean, Griffiths and Williams.
Ibid., 1928, lxv., 77.
9. Bennett and Ryle.
Guy's Hosp. Rep., 1921, lxxi., 286.
10. Bennett.
Brit. Med. Journ., 1923, ii., 275.
11. Butcher.
Quart. Journ. Med., 1926, xix., 455.
12. Duthie.
Quart. Journ. Med., 1927, xx., 265.
13. Lion, Bartle and Ellison.
New York Med. Journ., 1921, cxiv., 272.
14. Snapper.
Arch. des Malad. de l'Appareil Digest. et de la Nutrit., 1924, xiv., 677.
15. Paterson.
Lancet, 1924, i., 543.
16. Willcox.
Lancet, 1924, i., 544.
17. Lenhartz.
Deutsch. med. Woch., 1904, xxx., 412.
18. Lambert.
Amer. Journ. Med. Sci., 1908, cxxxv., 18.
19. Brown.
"*Physiological Principles in Treatment*," 5th edn., 1924, 86. Baillière, Tindall and Cox, London.
20. Sippy.
Journ. Amer. Med. Assocn., 1915, lxiv., 1,625.
21. Freezer, Gibson and Matthews.
Guy's Hosp. Rep. 1928, lxxviii., 191.
22. Hurst.
Brit. Med. Journ., 1928, ii., 779.
23. Hardt and Rivers.
Arch. Int. Med., 1923, xxxi., 171.
24. Hardy.
Lancet, 1928, i., 834.
25. Young.
Practitioner, 1928, cxx., 244.

CHAPTER VII

BASAL METABOLISM

It is hard to convey the meaning of the term "basal metabolism" in a few words. Metabolism means the tissue utilisation of food substances, and, since all these processes involve mainly oxidation, metabolism can be regarded as a process of slow combustion. Since combustion is attended with exothermic chemical reactions, it is possible to measure the combustion, *i.e.*, metabolism, by the amount of heat given out; again, since combustion is attended with the fixation of oxygen and liberation of CO_2, this process can be measured either by the oxygen consumption or by the CO_2 evolution. We have, therefore, two methods of measuring metabolism, either by the heat given out by the body or by the gaseous exchange in a given time.

The activities of the body can be restricted to such an extent that only the vital systems are at work. This condition of complete physical rest can be obtained by starving a person for twenty-four hours and keeping him in bed at complete mental and physical relaxation. Exogenous metabolism will be abolished and just sufficient energy will be expended by the body to keep the individual alive. The metabolism of such a patient is said to be "basal." *Basal metabolism is, therefore, the energy expenditure of an individual at complete physical and mental rest.* It can be recorded in a number of ways, none of which, however, is particularly straightforward. It cannot be expressed as calories given out per hour, or as c.c. of oxygen used per hour by an individual, since results so expressed would not be comparable one with another. The surface area of the person plays a very important part in basal metabolic determination. This

depends ultimately upon the fact that the smaller the weight the greater the area proportionately, and hence the mass of tissue will have to work at a greater rate to keep the area at a constant temperature. Thus it is well known that the smaller the animal the higher its metabolic rate. Again, the greater the area the more energy there is required to maintain it at a constant temperature.

From what has been said it can be seen that it would be incorrect to work out the basal metabolic rate per kilogram. It is usually expressed per square metre of body surface, either in calories or in c.c. of oxygen per unit of time. The age and sex of the patient also have a bearing upon the metabolic rate, and, consequently, must be allowed for. When the metabolism has been measured, the correct basal metabolism for a normal individual of that age and sex is looked up in a table, and the patient's result is expressed as a percentage increase or decrease, as the case may be.

Methods of Determination

These fall under two main headings—

Calorimetric.

Gasometric, which can be further sub-divided into two groups—

(*a*) Closed methods.

(*b*) Open methods.

Preparation of the Patient.—Preliminary treatment of the patient is necessary in order to ensure that he is at perfect physical and mental rest at the time of the observation. Thus exogenous metabolism and glandular secretions are reduced to a minimum by a previous sixteen to twenty-four hours' starvation. The patient is not allowed to read or talk, since these actions increase the metabolic rate. It is also essential to eliminate all element of fright or nervousness of the apparatus, which must be previously explained and demonstrated. It is stated that the slightest degree of apprehension throws out the results. The determination

should, if possible, be made at the bed-side, but if it has to be done in another room the patient must be wheeled there on a trolley and left for half an hour or so before the estimation is performed. These preliminaries are observed with the greatest care in America, but in England there seems to be a tendency to regard many of them as unessential. In some institutions basal metabolic determinations are performed on ambulatory cases, but the results obtained are of very doubtful value. If determinations are to be comparable, the rigid conditions laid down above must be adhered to.

Calorimetric Method.—Here the actual heat given out by the patient under the standard conditions is estimated by some form of calorimeter. Perhaps the best known instrument is that at the Russel Sage Institute, New York. The patient is placed in the calorimeter, the walls of which are kept at a constant temperature by an elaborate electrical device. The heat given out by the patient is absorbed by water running through pipes in the chamber. The accuracy of this particular calorimeter is said to be amazing, and it is claimed that if a definite weight of pure ether or alcohol be burned in the chamber the experimental measurement of the heat evolved corresponds exactly to the theoretical figure. Although this type of method is undoubtedly the most accurate, the expense of the apparatus forbids its general use, and, consequently, space cannot be afforded to describe it fully. A detailed account of this interesting work will be found in the various papers (1) and books on basal metabolism which are referred to at the end of this chapter.

Gasometric Methods.—In these methods the basal metabolic rate is measured by observing the amount of oxygen absorbed during a certain time. Alternatively, the same result may be arrived at by working out the quantity of CO_2 exhaled, although this type of method is not in extensive use.

(*a*) *The Closed Methods.*—These require some form of special apparatus, and the importance of having a really reliable instrument cannot be over-emphasised. Many of

those on the market are inaccurate and useless, mainly owing to bad graduation and to inefficient CO_2 absorption. A new apparatus should always be tested from both these points of view. The principle of this type of apparatus is as follows :—

It consists of a reservoir like a small gasometer, with a water seal. This is filled with oxygen, and is connected in series by means of tubes with a mouthpiece and a soda-lime container. The tubes are fitted with valves in such a manner that the patient inspires oxygen through the mouthpiece from the cylinder and expires through the soda-lime chamber into the reservoir again. The floating bell of the oxygen reservoir is fitted with a dial arrangement which records the rise or fall of the upper segment, thus indicating changes in volume. The dial reads off in cubic centimetres. Before use the reservoir is filled with oxygen and the apparatus is tested for leaks. The patient then breathes through the mouthpiece, which is disconnected, and the volume is read off on the dial. The patient's nose must be clipped. When he is comfortable and breathing naturally, the mouthpiece is connected with the apparatus and the time is taken. The patient is now taking oxygen from the reservoir into his lungs and is breathing out a mixture of oxygen and CO_2 into the soda-lime chamber. Here the latter gas is absorbed and the residual oxygen is passed on to the chamber. The volume will naturally be diminished. The decrease in volume is read off at five-minute intervals, and when some degree of constancy in the figure is observed the experiment is terminated. The mean of the five-minute readings gives the amount of oxygen absorbed in that time. It is essential to make several readings, since if one be taken immediately after an irregular inspiration or expiration, the error will be great. The volume is then reduced to standard temperature and pressure by means of tables supplied with the apparatus, and the metabolic rate is calculated in the manner described later. Some of the more expensive types are fitted with

circulation fans, but the cheaper models are quite satisfactory. These methods possess the great advantage that no knowledge of gas analysis is required and they are very easy to perform.

(*b*) *The Open Methods.*—These will be described in greater detail, since they are used extensively in this country.

The apparatus required consists of:—

Douglas bag with mouthpiece and valves (2).
Haldane gas analysis apparatus (3).
Gas meter.
Sampling tubes.

A very neat and compact outfit can be purchased from Messrs. Siebe, Gormans & Co.

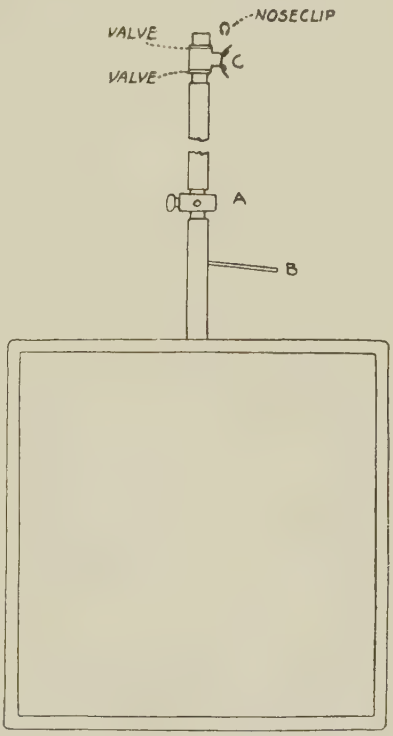


Fig. 6.—The Douglas Bag.

The bag (see Fig. 6) is made of rubber-coated cloth, and is fitted with a tube bearing a two-way tap A and side tube B. The far end of A is connected with the mouthpiece C, bearing valves. The tap A can be arranged so that it puts the bag in communication with the mouthpiece, or else shuts off the bag and communicates the mouthpiece with the exterior. To collect the sample the side tube B is clipped, and the bag is emptied, either by careful rolling up or by a vacuum pump. The tap A is then turned so that the bag is shut off and the

mouthpiece is open to the outside air. The patient then fixes the mouthpiece in position and adjusts the nose clip, so that all his respirations are through the apparatus. Some forms of mouthpiece are fitted with a mask in order to ensure that no air is obtained other than through the valves, which are so arranged that the patient breathes in ordinary air

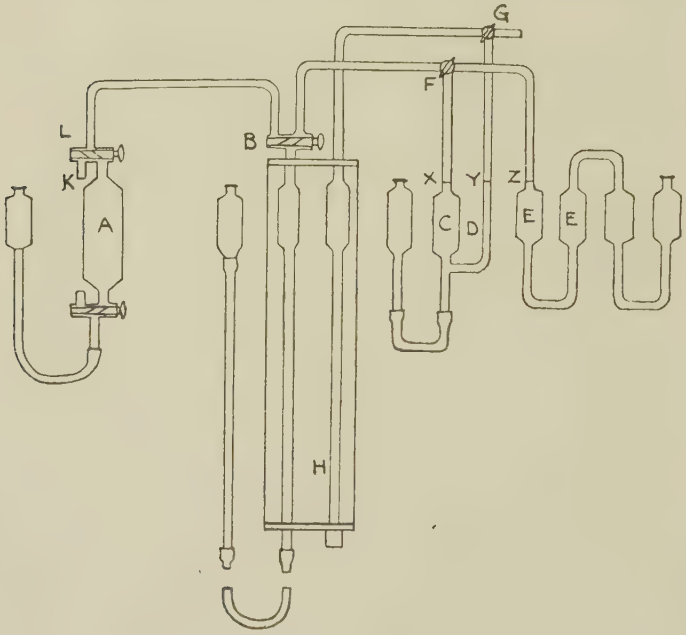


Fig. 7.—Haldane Gas Analysis Apparatus with Sampler.

through the top of the mouthpiece and out into the tube connecting with the bag. Owing to the position of the tap A, his expirations pass out into the air. When the patient is breathing naturally and calmly the time is noted and the tap A is turned so that the expirations collect in the bag. This is allowed to go on until the bag is moderately full, usually about ten minutes, when the tap A is adjusted so as to close the bag and the experiment is terminated. The time of

turning the tap is taken, and the bag is removed to the laboratory for analysis of the contents. The apparatus required for the analysis is shown in diagrammatic form above (see Fig. 7).

It consists of a sampling tube A connected with a mercury reservoir and fitted with two-way taps at either end. One of the exit tubes at the upper end is connected to the two-way tap B of the burette of the gas-analysis apparatus. This burette is graduated and is connected with a movable mercury reservoir. The other exit from the burette is connected to other parts of the apparatus, as shown in the diagram. C contains 10% KOH, and is fitted with a movable reservoir on the left, and the side tube D is in connection with a blind control tube H, of exactly the same volume as the gas burette. By means of the three-way tap G it is possible to make D and H communicate, or to make them both communicate with the outside air.

The bulbs E contain a 10% solution of pyrogallic acid in saturated KOH solution. By means of the tap F it is possible to put the gas burette into communication with either the potash bulb C or the pyrogallol bulbs E.

Before use the bulbs C and E are filled with their respective solutions, and the quantities are so arranged that, with all the taps open to the exterior, the levels come to the marks X and Y and Z on the tubes.

Before an analysis can be performed the apparatus must be cleared of oxygen and CO_2. This is effected by filling the burette with air and turning the taps B and F so that the burette is put into communication with the potash bulb C. The mercury reservoir belonging to the burette is raised and lowered frequently, with the result that the contained gas is blown over on to the potash, where the CO_2 is absorbed. This is done until a constant reading is obtained on the burette, when the levels of potash are at X and Y, as they were at the outset of the experiment. All the CO_2 has now been absorbed, and it remains to get rid of the oxygen. This is done by

turning the tap F so that the burette communicates with the pyrogallol bulbs E. On raising the mercury reservoir belonging to the burette, the CO_2-free air is passed into two pyrogallol absorption bulbs E, where oxygen is absorbed. The gas is passed rapidly backwards and forwards until the volume recorded is constant, readings being taken with the pyrogallol level at Z, the mark on the tube. All the oxygen in the apparatus has now been absorbed, except for a little contained in the air trapped between F and X. The tap F is then turned to communicate the burette with C, and the mercury reservoir is rapidly raised and lowered in order to mix the specimens. The tap is then turned so that the mixture can be passed into the pyrogallol bulb, where the remaining oxygen can be absorbed.

This process is carried on until a constant reading is obtained. The apparatus has now been cleared of CO_2 and oxygen and is ready for a determination.

Analysis of Specimen from Bag.—The sampler A is filled with mercury up to the end of the free tube L. This is done by setting the lower tap so that it puts the mercury into communication with the side tube, which is filled with mercury. The tap is then turned so that it communicates with the sampler.

By this means the tap is sealed with mercury and the possibility of air bubbles is excluded.

The clip on the side tube of the Douglas bag is slackened, and a very small quantity of air is run out of the bag. The free end of the tube is immediately attached to L, and the mercury reservoir is lowered. A sample of air is thereby sucked into the sampler. The mercury reservoir is repeatedly raised and lowered, in order that a good specimen be obtained. After thorough mixing has been ensured the reservoir is lowered so that the sampler is just full of air. Both taps are then shut. The connecting tube between the gas burette and the sampler is then put in position by means of rubber connections. The tap G is then turned so that H and D are

open to the air. The potash levels are adjusted to X and Y by moving the mercury and potash reservoirs respectively. G is then closed so that D and H form a closed space and the tap B is reversed, thus connecting the burette with the sampler. The upper tap of the sampler is adjusted to connect L with the open tube K. The mercury reservoir belonging to the burette is raised, thus driving out the O_2- and CO_2-free gas over the connecting tube and out at K. When the whole system is full of mercury up to K the tap of the sampler is reversed, the lower one is opened and the reservoir of the gas burette is depressed.

This results in the gas contained in the sampler being sucked over into the burette. The reservoir is repeatedly raised and lowered to ensure thorough mixing. When this has been obtained, the two mercury reservoirs are so adjusted that about 20 c.c. of gas have been sucked into the burette and the levels of mercury inside and outside the burette and sampler are respectively the same. The tap of the sampler and the tap B are closed, and a little time is allowed for the gas to cool to the temperature of the water jacket surrounding the burette. The taps B and F are then turned to put the burette into communication with C. There will be an immediate change in the levels X and Y, which must be brought back to their former position by altering the heights of the mercury and potash reservoirs respectively. When this has been accomplished, the volume on the burette can be read off, since the contents will now be at atmospheric pressure, the levels X and Y having been previously adjusted with the tap G open. The volume of gas is noted and the CO_2 is absorbed in exactly the same manner described above. The final readings, however, must only be taken with the potash levels on the marks X and Y. When two constant readings have been obtained, the oxygen can be absorbed as already described. Again the final readings must be made with the burette in communication with the potash bulbs alone and with the levels on the marks X and Y. The barometric pres-

sure and room temperature are taken. The volume of gas in the bag is measured by passing it through a meter, and to that figure is added the volume of the sample (75 c.c.). It is a good plan to analyse two samples of 75 c.c. Given the patient's height, weight, age and sex, the basal metabolic rate can be calculated.

Calculation.—Although only elementary mathematics are employed, the calculation is somewhat involved. It can, however, be divided into a series of steps.

1. Calculation of percentage composition of the expired air, together with respiratory quotient.

Burette Readings

First reading . . .	20·00
Second reading . . .	19·19
Therefore CO_2 absorbed .	0·81
	= 4·05%
Second reading of burette .	19·19
Third reading . . .	15·89
Therefore oxygen absorbed .	3·30
	= 16·5 %

The gas in the bag had, therefore, the following composition :—

CO_2 . . .	4·05%
O_2 . . .	16·5 %
N_2 . . .	79·45%

Composition of atmospheric or inspired air :—

CO_2 = negligible (0·03%)
O_2 = 20·94%
N_2 = 79·06%

It would appear, at first sight, that the amount of O_2 absorbed per 100 c.c. would be given by subtracting the percentage of oxygen in the expired air from that in the inspired air. This, however, cannot be done, since, as can be seen from the above calculation, the percentage of nitrogen in the two specimens has changed, although the absolute amount

is unaltered. Before subtracting, it is necessary to calculate to what percentage of oxygen in the inspired air the nitrogen percentage in the expired air would correspond. This may be done in the following manner, or by means of Table 1, p. 229.

79·06% of N_2 corresponds to 20·94% of oxygen.

Therefore

$$79{\cdot}45\% \text{ of } N_2 \text{ corresponds to } \frac{20{\cdot}94}{79{\cdot}06} \times 79{\cdot}45\% \text{ oxygen}$$
$$= 21{\cdot}05.$$

In Table 1 will be found the O_2 equivalent of various nitrogen contents.

The percentage amount of oxygen absorbed was therefore the corrected O_2% in inspired air — O_2% in expired air.

$$= 21{\cdot}05 - 16{\cdot}5\%.$$
$$= 4{\cdot}55\%.$$

The CO_2 absorbed $= 4{\cdot}05 - 0{\cdot}03 = 4{\cdot}02\%$.

The respiratory quotient is therefore $\frac{CO_2}{O_2} = \frac{4{\cdot}02}{4{\cdot}55} = 0{\cdot}88$.

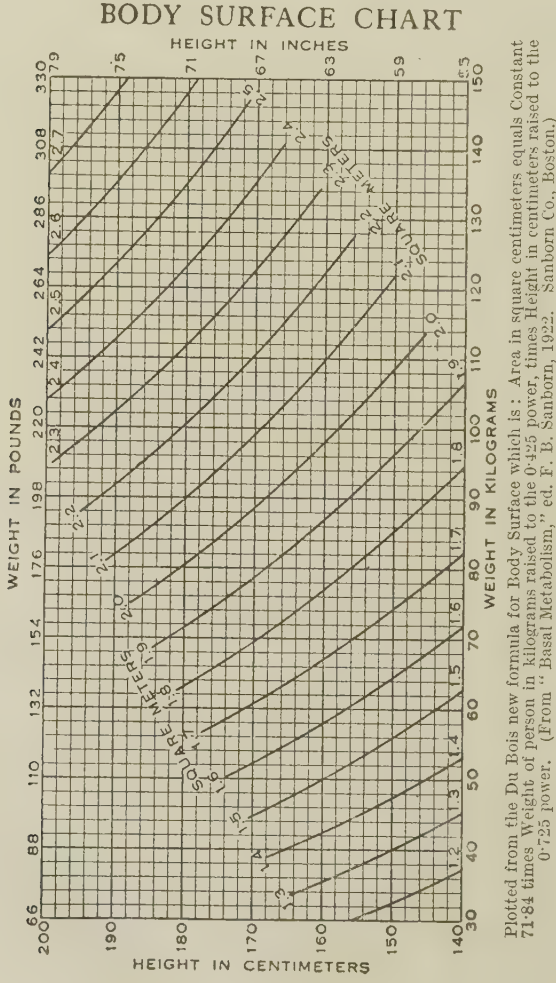
The time taken for the experiment was fifteen minutes, in which time 100 litres collected in the bag. The barometer stood at 747 mm., and the temperature was 20° C. The volume of gas must be reduced to standard conditions, 0° C. and 760 mm., and a correction must be applied for the water vapour tension, since the air in the bag will be saturated. The barometer reading will have to be corrected for expansion of the scale. This can be done by obtaining the temperature factor from Table 3, p. 230. The factor is in this case 2·39 mm. The correction for the aqueous tension, as read off from Table 2, p. 229, is 17·4.

Therefore the correct pressure $= 747 - (17{\cdot}4 + 2{\cdot}39)$.
$= 727{\cdot}21$ mm.

The reduction to 0° C. and 760 mm. pressure is accomplished by obtaining a factor from Table 4, p. 230. Since

the temperature is 20° C., and the corrected pressure is 727·21 mm., the factor, as calculated from Table 4, p. 230, will be found to be 0·892.

Therefore the volume at 0° C. and 760 mm. Hg = 89·2 litres.



Plotted from the Du Bois new formula for Body Surface which is: Area in square centimeters equals Constant 71·84 times Weight of person in kilograms raised to the 0·425 power, times Height in centimeters raised to the 0·725 power. (From "Basal Metabolism," ed. F. B. Sanborn, 1922. Sanborn Co., Boston.)

The amount of oxygen used up was 4·55% of the volume,

i.e., $\frac{4{\cdot}55}{100} \times 89{\cdot}2$ litres.

= 4·0586 litres per fifteen minutes.
= 270·5 c.c. per minute.
= 16·2 litres per hour.

For some purposes it is necessary to work out the area of the patient. This can be done from the accompanying body surface chart. The subject, a male aged 18, weighed 61 kg. and the height was 144 cm. The area was therefore 1·5 sq. metres. The gas exchange was therefore :—

$\frac{270{\cdot}5}{1{\cdot}5}$ = 180 c.c. per minute per sq. metre

or

$\frac{16{\cdot}2}{1{\cdot}5}$ = 10·8 litres of oxygen per hour per sq. metre.

With the data now at our disposal it is possible to work out the final results in one of two ways ; the first depends merely on the gas exchange, while the second depends upon the calculation of the number of calories per square metre per hour given off by the patient.

First Method.—By reference to Table 5, p. 231, the normal oxygen consumption per minute can be ascertained. For a man of that weight and height it should be 196 c.c. of oxygen per minute. Corrections must now be made for age and sex according to the following scheme :—

Males.—20 to 50, no correction.
16 to 18, add 1% to volume in table.
18 to 20, add 5% to volume in table.
50 to 60, subtract 4% from volume in table.
60 to 70, subtract 7% from volume in table.

Females.—Calculate as male of same age and subtract 7%.

The patient's basal metabolic rate is then expressed as a percentage increase or decrease on the standard corrected volume. Since the subject was a male, aged 18, 1% must be added to the standard figure of 196 c.c. obtained from

Table 5, p. 231, making it 198 c.c. The patient's oxygen consumption, however, was 270 c.c. There was therefore an increase of 72 c.c. above the standard of 198 c.c. The percentage increase in basal metabolic rate was therefore $\frac{72}{198} \times 100 = +36\%$.

Second Method.—The gas exchange can be converted into caloric values provided that the respiratory quotient is known. Table 6, p. 234, gives the caloric values of 1 litre of oxygen at various respiratory quotients. Calculation of results by this method is known as *indirect calorimetry*. It has been shown that the oxygen consumption per hour is 16·2 litres, and that the respiratory quotient is 0·88. By reference to Table 6, p. 234, it can be found that at this quotient, 1 litre of oxygen corresponds to 4·9 calories. The total number of calories per hour was therefore $16{\cdot}2 \times 4{\cdot}9 = 79{\cdot}38$ calories. The area of the patient has been previously calculated as 1·5 square metres. The patient therefore radiated $\frac{79{\cdot}38}{1{\cdot}5}$ calories per square metre, which equals 52·9 calories. By reference to Table 7, p. 235, we see that a normal male aged 18 should give out 39·2 calories per square metre. There is, therefore, an increase of 13·7 over the normal of 39·2; *i.e.*, $\frac{13{\cdot}7}{39{\cdot}2} \times 100\% = 35\%$.

By the first method the result came to 36%. The small difference is due to approximations and inaccuracies in the estimation of surface area, and in the relation between calories and oxygen consumption.

Interpretation of Results.—The first question to consider is the accuracy of the results as furnished by any of the methods described.

Practically all observers agree that it is possible to have a plus or minus error of 15%. That is to say, nothing of diagnostic importance can be deduced from figures 15% removed from the normal. With the calorimetric methods,

almost the only source of error lies in the patient moving, etc., and thus raising his metabolic rate. This can account for very considerable elevations above the normal. Thus the B.M.R. of a patient sitting up was found by Emmes and Riche (4) to be 7·6% higher than when he was lying down. An uncomfortable position, attended with slight muscular action, was found by Magnus-Levy (5) to cause an increase of 20% or over. The discomfort of a full bladder will raise the rate by over 20%. Again, if the patient be nervous at the time of the determination, the conditions will be far from basal. A meal increases the rate by 10 to 15%, and the effect may last for twelve hours. Rubner (6) demonstrated that an imperceptible air current on an exposed surface greatly increased the metabolic rate.

In view of these disturbing factors, we always estimate the B.M.R. several times on the same patient, using the Douglas bag method. The estimations are continued until two results are obtained which agree within 5%. Experience has shown that it is very unwise to place any reliance on a single determination, since there are so many points in the estimation where errors may creep in. Even although the individual errors may be small, the sum total in the end result will be considerable. The closed methods possess the advantage that four or five determinations may be made at one sitting, and, consequently, the result is bound to be more accurate than a single determination by the Douglas bag method. On the other hand, a series of separate determinations by the latter technique are, perhaps, preferable.

Despite the apparent sensitiveness of metabolism to various extraneous factors, and the difficulty attending their exclusion, it is claimed that the estimation is of the greatest value. Investigation of disease by this method is said to yield valuable information in cases showing disturbances of the endocrine organs, and more especially thyroid and pituitary conditions.

Thyroid Disturbances.—In exophthalmic goitre the basal metabolic rate is greatly increased. The increase also varies

proportionately to the severity of the condition, and, consequently, cases can be classified according to what increase they show in their basal metabolic rate. Thus C. H. Frazier and Adler (7) classify their cases as follows :—

Group I.	.	B.M.R. from 10 to 20% increase.
,, II.	.	B.M.R. ,, 20 to 40% ,,
,, III.	.	B.M.R. ,, 40 to 60% ,,
,, IV.	.	B.M.R. ,, 60% upwards increase.

The treatment varies for each group. Frazier recommends subtotal thyroidectomy for Groups I. and II., bilateral ligation of the superior thyroid arteries for Group III., and unilateral ligature for Group IV.

Many American authorities state that they rely almost entirely on the B.M.R. determination for indications for surgical treatment. Sir James Berry (8), however, in this country, considers that the cardiac side of the disease requires just as careful consideration as the metabolic. He relies upon clinical and electro-cardiographic examination, together with the metabolic, for indications as to treatment (see p. 260). This observer also considers that extensive operative interference is very unwise in cases with a basal metabolic rate of over 40% increase.

If the basal metabolic rate be increased by over 50%, the application of radium or X-rays has been reported to give very good results. This form of treatment may also be applied to cases unsuited to surgical treatment. After the treatment there is an immediate rise in the rate, followed by a fall. Thus Aub and Means (9) report that each application was followed roughly by a fall of 10%. The number of applications required for a cure, however, is numerous, and the treatment must be prolonged for many months, or even over a year. The metabolic determination is of great value in checking any form of treatment, either surgical, X-ray, or medical. In hypothyroidism the rate is decreased down to 20 to 40% below the normal. The rate can be restored to

normal by the administration of thyroid extract. This reduction in hypothyroidism is very useful for diagnostic purposes. Unfortunately, hypothyroidism is not the only condition which can effect a lowering in the basal metabolic rate. Thus prolonged under-nutrition, the terminal stages of various diseases, such as chronic nephritis, diabetes, and particularly hypopituitarism, are associated with a decreased metabolism.

A method for calculation of the B.M.R. in hyperthyroidism from a formula, in which only the pulse rate and pulse pressure have to be determined, has been introduced by Read (10).

Read's Formula for Calculating Basal Metabolism in Hyperthyroidism.—Read (10) in 1922, introduced a formula to establish a relationship between the pulse rate and pulse pressure and the basal metabolic rate in cases of hyperthyroidism. He found, as the result of observations made on a series of 300 cases, that by estimating the pulse rate and pulse pressure under "basal" conditions the basal metabolic rate could be calculated with an error of only 10% in 60% of cases, and of 20% in 91% of cases. This error may be either a plus or minus one.

The formula is as follows: B.M.R. = 0·683 (P.R. + 0·9 P.P.) – 71·5, where P.R. = "basal" pulse rate, and P.P. = "basal" pulse pressure, *i.e.*, the difference between the systolic and diastolic blood pressure observed when the patient is kept under basal conditions such as are required for the estimation of the B.M.R. Generally speaking, in the absence of some gross cardio-vascular lesion such as aortic reflux or arterio-venous aneurysm, an increase in pulse pressure implies an acceleration of blood flow through the tissues. Harris (11) considers that a combination of an increase in pulse rate and pulse pressure only occurs in hyperthyroidism. Davies and Eason (12) have recorded a series of observations on basal metabolism, pulse rate and pulse pressure. They find that an increased pulse pressure is a

characteristic feature of hyperthyroidism, whereas in uncomplicated hypothyroidism there is a fall in pulse pressure. We have tested the formula on several occasions, and find that the figure obtained usually corresponds fairly closely with the B.M.R. as actually estimated. The following is an example from one of our cases :—

Male with Graves' disease. Auricular fibrillation removed by quinidine. P.R. = 95. P.P. = 75, *i.e.* (135–60).

$$\text{B.M.R.} = 0{\cdot}683\ (95 + 0{\cdot}9 \times 75) - 71{\cdot}5$$
$$= +\ 39{\cdot}4\%$$

The basal metabolic rate as estimated by the Douglas bag method was + 40%.

After double ligation of the superior thyroid arteries the basal metabolic rate as calculated by Read's formula fell to + 22·7%, and as estimated by the Douglas bag method it was + 21%. The pulse rate on this occasion was 75, the systolic blood pressure 130, and the diastolic blood pressure 60 mm. Hg.

Pituitary Disturbances.—The basal metabolic rate is decreased in hypopituitarism, although not so markedly as in deficient thyroid function. The decrease is usually not much greater than — 13 to — 30%.

That this is not due to thyroid disturbances can only be determined by clinical examination, thus noting the absence of signs of myxœdema, and by giving a dose of pituitrin. If the decrease be due to pituitary inefficiency, the injection will be followed by an increase in the basal metabolic rate, which is not obtained if the thyroid alone be at fault. Pituitary extract is said to enhance the action of thyroid extract upon the basal metabolic rate. The estimation of basal metabolic rate is also stated to be of the greatest value in various other conditions. Thus in fevers, Eugene F. Du Bois (13) holds that its estimation is of value from the point of view of prognosis, and, to a certain extent, for treatment purposes.

The main uses of basal metabolism estimation can be summarised as follows :—

Determining the course of treatment to be adopted in a case of hyperthyroidism.

Checking that treatment.

Diagnosis of thyroid and pituitary lesions.

Investigation of cases of obesity to determine the endocrine factor, if any.

Table 1

The Percentage of Oxygen which is Equivalent to the Nitrogen found in the Expired Air

To obtain the nitrogen in the expired air, add the percentage of CO_2 and O_2 found and subtract the sum from 100. The table gives the percentage for O_2 corresponding to this figure.

% N_2	78·7	78·8	78·9	79·0	79·1	79·2	79·3
% O_2	20·86	20·88	20·90	20·93	20·96	20·98	21·01
% N_2	79·4	79·5	79·6	79·7	79·8	79·9	80·0
% O_2	21·04	21·07	21·10	21·12	21·14	21·16	21·19
% N_2	80·1	80·2	80·3	80·4	80·5	80·6	—
% O_2	21·22	21·25	21·28	21·31	21·35	21·38	—

(From Macleod. "Physiology and Biochemistry in Modern Medicine," 1918. Kimpton, London.)

Table 2

Tension of Aqueous Vapour in Millimetres of Mercury

To obtain the dry barometer pressure, subtract the mm. Hg corresponding to the temperature of the air from the barometer pressure at the time of the experiment.

Temp.	15°	16°	17°	18°	19°	20°	21°	22°	23°	24°	25°
Mm.	12·7	13·5	14·4	15·4	16·3	17·4	18·5	19·7	20·9	22·2	23·5

(From Macleod. "Physiology and Biochemistry in Modern Medicine," 1918. Kimpton, London.)

Table 3

Temperature Corrections to Reduce Readings of a Mercurial Barometer with a Brass Scale to 0° *C.*

Subtract the appropriate quantity as found in table from the height of the barometer. The table is for a barometer with a brass scale, and the values are a little lower (about 0·2 mm.) than for the glass scale. The corrections for intermediate temperatures can be approximated.

Temp.	700 mm.	710 mm.	720 mm.	730 mm.	740 mm.	750 mm.	760 mm.	770 mm.
15° . .	1·69	1·72	1·74	1·77	1·79	1·81	1·84	1·86
20° . .	2·26	2·28	2·32	2·36	2·39	2·42	2·45	2·48
25° . .	2·83	2·87	2·91	2·95	2·99	3·03	3·07	3·11

(From Macleod. " Physiology and Biochemistry in Modern Medicine," 1918. Kimpton, London.)

Table 4

Table for Reducing Gaseous Volumes to Normal Temperature and Pressure

The observed volume, when multiplied by the factor corresponding to the temperature and pressure, will give the volume of the expired air reduced to 0° C. and 760 mm.

Mm.	15°	16°	17°	18°	19°	20°	21°	22°	23°	24°	25°
720	·898	·894	·891	·888	·885	·882	·880	·877	·873	·870	·867
730	·910	·907	·904	·901	·897	·894	·891	·888	·885	·882	·879
740	·922	·919	·916	·913	·910	·907	·904	·901	·897	·894	·891
750	·935	·932	·928	·925	·922	·919	·916	·913	·910	·907	·904
760	·947	·944	·941	·938	·934	·931	·928	·925	·922	·919	·916
770	·960	·957	·953	·950	·948	·945	·940	·936	·933	·930	·927

(From Macleod. " Physiology and Biochemistry in Modern Medicine," 1918. Kimpton, London.)

Table 5

Showing the amount of oxygen absorbed, under basal conditions, and at 0° C. and 760 mm. pressure, by individuals in relation to their height and weight

HEIGHT IN CENTIMETERS

WEIGHT IN KILOGRAMS	140	145	150	155	160	165	170	175	180	185	190	WEIGHT IN POUNDS
30	142	145	149	152	156	159	163	166	170	173	177	66
1	144	147	151	154	158	161	165	168	172	175	179	68
2	145	149	153	156	160	163	167	170	174	177	181	70
3	147	151	155	158	162	165	169	173	177	180	184	73
4	149	153	157	160	164	167	171	175	179	182	186	75
5	151	155	159	162	166	170	174	177	181	184	188	77
6	153	157	161	164	168	172	176	179	183	187	191	79
7	155	159	163	166	170	174	178	182	185	189	193	81
8	156	161	164	168	172	176	180	184	188	191	195	84
9	158	162	166	170	174	178	182	186	190	194	198	86
40	159	164	168	172	176	180	184	188	192	196	200	88
1	161	165	169	174	179	182	186	190	194	198	202	90
2	162	167	171	176	181	184	188	192	196	200	204	92
3	164	169	173	178	182	185	189	194	198	202	206	95
4	165	170	175	180	184	187	191	196	200	204	208	97
5	167	172	177	181	186	189	193	198	201	205	210	99
6	169	174	179	183	188	191	195	200	203	207	212	101
7	171	176	180	184	189	192	197	202	205	209	214	103
8	172	177	182	186	191	194	199	204	207	211	216	106
9	174	179	183	188	192	196	201	206	209	213	218	108
50	176	181	185	189	193	197	202	207	211	215	220	110
1	178	183	186	191	195	199	204	209	212	217	221	112
2	179	184	188	192	196	200	205	211	214	219	223	114
3	181	186	189	194	198	202	207	212	216	220	225	117
4	182	187	191	195	199	203	208	214	217	222	227	119
5	184	188	193	197	201	205	210	216	219	224	228	121
6	185	189	194	199	203	207	212	218	221	226	230	123
7	186	191	196	200	205	209	213	219	222	228	232	125
8	188	192	197	202	206	210	215	221	224	229	233	128
9	189	194	199	203	208	212	216	222	226	231	235	130
60	190	195	200	204	209	213	217	223	228	233	237	132
1	191	196	201	206	211	215	219	225	230	235	239	134
2	192	198	202	207	212	216	220	226	231	236	240	136
3	193	199	203	209	214	218	222	228	233	238	242	139
4	195	200	205	210	215	219	223	229	234	239	243	141
5	196	201	206	212	217	221	225	231	236	241	245	143
6	197	202	207	213	218	222	227	232	237	242	247	145
7	199	204	209	215	220	224	228	234	239	244	249	147
8	200	205	210	216	221	225	230	235	240	245	250	150
9	201	207	212	218	222	227	231	237	242	247	252	152
70	202	208	213	218	223	228	233	238	243	248	253	154
1	203	209	214	219	224	229	235	240	245	250	255	156
2	204	210	216	220	225	231	236	241	246	251	256	158
3	206	212	217	222	227	232	238	243	248	253	258	161
4	207	213	219	223	228	234	239	244	249	254	259	163
5	208	214	220	224	229	235	241	246	251	256	261	165
6	209	215	221	225	230	236	242	247	252	257	263	167
7	210	216	222	227	232	238	244	249	254	259	264	169
8	212	218	224	228	233	239	245	250	255	260	266	172
9	213	219	225	230	235	240	246	251	257	262	267	174
80	214	220	226	231	236	241	247	253	258	263	268	176
1	215	222	227	232	237	242	248	254	259	264	270	178
2	217	223	228	234	239	244	250	256	261	266	271	180
3	218	225	230	235	240	245	251	257	262	267	273	183
4	220	226	231	237	242	247	253	259	264	269	274	185
5	221	227	233	238	243	248	254	260	265	270	276	187
6	222	228	234	239	244	249	255	261	266	271	277	189
7	223	229	235	240	246	251	256	262	268	273	279	191
8	224	230	236	242	247	252	258	264	269	274	280	194
9	225	231	237	243	248	254	259	265	271	276	282	196
	55	57	59	61	63	65	67	69	71	73	75	

HEIGHT IN INCHES

Table 5 (continued)

HEIGHT IN CENTIMETERS

WEIGHT IN KILOGRAMS	150	155	160	165	170	175	180	185	190	195	200	WEIGHT IN POUNDS
90	238	244	249	255	260	266	271	276	282	287	292	198
1	239	245	250	256	261	267	272	277	283	288	293	200
2	240	246	251	257	262	268	273	278	284	289	294	202
3	241	247	252	258	264	269	275	280	286	291	296	205
4	242	248	253	259	265	270	276	281	287	292	297	207
5	243	249	254	260	266	271	277	282	288	293	298	209
6	244	250	255	261	267	272	278	283	289	294	299	211
7	245	251	256	263	269	274	279	284	290	295	300	213
8	246	252	258	264	270	275	281	286	292	297	302	216
9	247	253	259	266	271	277	282	287	293	298	303	218
100	248	254	260	267	272	278	284	289	294	299	304	220
1	249	255	261	268	273	279	285	290	295	300	305	222
2	250	256	262	269	274	280	286	292	297	302	307	224
3	251	257	263	270	275	281	288	293	298	303	308	227
4	252	258	264	271	276	282	289	294	299	304	309	229
5	253	260	266	272	278	284	290	296	301	306	311	231
6	254	261	267	273	279	285	291	297	302	307	312	233
7	255	262	268	274	280	286	293	298	303	308	313	235
8	256	263	269	275	281	287	294	300	305	310	315	238
9	257	264	270	276	282	288	295	301	306	311	316	240
110	258	265	271	277	283	289	296	302	307	312	317	242
1	259	266	272	278	284	290	297	303	308	313	318	244
2	260	267	273	279	285	291	298	304	309	315	320	246
3	261	268	274	280	286	293	299	305	310	316	321	249
4	262	269	275	281	287	294	300	306	312	317	322	251
5	263	270	276	283	289	295	302	308	313	319	324	253
6	264	271	277	284	290	296	303	309	314	320	325	255
7	265	272	278	285	291	298	304	310	315	321	326	257
8	266	273	279	286	292	299	305	311	317	323	328	260
9	267	274	280	287	293	300	306	312	318	324	329	262
120	268	275	281	288	294	301	307	313	319	325	330	264
1	269	276	282	289	295	302	308	314	320	326	331	266
2	270	277	283	290	296	303	309	315	321	327	332	268
3	271	278	284	291	297	304	310	316	322	328	333	271
4	272	279	285	292	298	305	311	317	323	329	335	273
5	272	279	285	292	299	306	312	319	325	331	336	275
6	273	280	286	293	300	307	313	320	326	332	337	277
7	274	281	287	294	301	308	314	321	327	333	339	279
8	275	282	288	295	302	309	315	322	328	334	340	282
9	276	283	289	296	303	310	316	323	329	335	341	284
130	277	284	290	297	304	311	317	324	330	336	342	286
1	278	285	291	298	305	312	318	325	331	337	343	288
2	279	286	292	299	306	313	319	326	332	338	344	290
3	280	287	293	300	307	314	320	327	333	339	345	293
4	281	288	294	301	308	315	321	328	334	340	346	295
5	281	288	294	302	309	316	323	329	335	341	347	297
6	282	289	295	303	310	317	324	330	336	342	348	299
7	283	290	296	304	311	318	325	331	337	343	349	301
8	284	291	297	305	312	319	326	332	338	344	350	304
9	285	292	298	306	313	320	327	333	339	345	351	306
140	286	293	299	307	314	321	328	334	340	346	352	308
1	287	294	300	308	315	322	329	335	341	347	353	310
2	288	294	301	309	315	323	329	336	342	348	354	312
3	289	295	302	310	316	324	330	337	343	349	355	315
4	289	296	303	311	317	325	331	338	344	350	356	317
5	290	297	304	312	318	326	332	339	345	351	357	319
6	291	298	305	313	319	327	333	339	346	352	358	321
7	291	299	306	314	320	328	334	340	346	353	359	323
8	292	300	307	315	321	329	335	341	347	354	360	326
9	293	301	307	315	322	329	336	342	348	355	361	328
	59	61	63	65	67	69	71	73	75	77	79	

HEIGHT IN INCHES

Table 5 (continued)

NORMAL STANDARDS OF BASAL METABOLISM FOR BOYS AND GIRLS OF WEIGHTS 20 TO 38 KILOS*

(Ages 6 to 12 years approximately)

Weight is determining factor for basal metabolism of boys and girls

Body Weight (without clothing)		Heat Production in Calories per 24 hours		Oxygen Consumption in Cubic Centimeters per minute	
Kilos	Pounds	Boys	Girls	Boys	Girls
20.0	44	860	805	124	116
20.5	45	873	818	126	118
21.0	46	885	830	127	120
21.5	47	898	842	129	121
22.0	48	910	855	131	123
22.5	50	925	867	133	125
23.0	51	940	880	135	127
23.5	52	953	890	137	128
24.0	53	965	900	139	130
24.5	54	978	915	141	132
25.0	55	990	930	143	134
25.5	56	1005	940	145	135
26.0	57	1020	950	147	137
26.5	58	1033	962	149	139
27.0	59	1045	975	150	140
27.5	61	1058	987	152	142
28.0	62	1070	1000	154	144
28.5	63	1080	1010	155	145
29.0	64	1090	1020	157	147
29.5	65	1103	1032	159	149
30 0	66	1115	1045	161	150
30 5	67	1127	1058	162	152
31.0	68	1140	1070	164	154
31.5	69	1150	1080	166	155
32.0	70	1160	1090	167	157
32.5	72	1170		168	
33.0	73	1180		170	
33.5	74	1190		171	
34.0	75	1200		173	
34.5	76	1210		174	
35.0	77	1220		176	
35.5	78	1230		177	
36.0	79	1240		179	
36.5	80	1248		180	
37.0	81	1255		181	
37.5	83	1265		182	
38.0	84	1275		184	

* Predicted standards. Prepared by editor from Table 36 in Benedict and Talbot's "Metabolism from Birth to Puberty." Carnegie Institute of Washington, Washington, D C., 1921.

(From Sanborn. "Basal Metabolism," 1922, Sanborn Co., Boston.)

Table 6

R.Q.	Calories for 1 litre O_2	Relative Calories Consumed as	
		Carbohydrate per cent.	Fat per cent.
0·70	4·686	0	100
0·71	4·690	1·4	98·6
0·72	4·702	4·8	95·2
0·73	4·714	8·2	91·8
0·74	4·727	11·6	88·4
0·75	4·739	15·0	85·0
0·76	4·752	18·4	81·6
0·77	4·764	21·8	78·2
0·78	4·776	25·2	74·8
0·79	4·789	28·6	71·4
0·80	4·801	32·0	68·0
0·81	4·813	35·4	64·6
0·82	4·825	38·8	61·2
0·83	4·838	42·2	57·8
0·84	4·850	45·6	54·4
0·85	4·863	49·0	51·0
0·86	4·875	52·4	47·6
0·87	4·887	55·8	44·2
0·88	4·900	59·2	40·8
0·89	4·912	62·6	37·4
0·90	4·924	66·0	34·0
0·91	4·936	69·4	30·6
0·92	4·948	72·8	27·2
0·93	4·960	76·2	23·8
0·94	4·973	79·6	20·4
0·95	4·985	83·0	17·0
0·96	4·997	86·4	13·6
0·97	5·010	89·8	10·2
0·98	5·022	93·2	6·8
0·99	5·034	96·6	3·4
1·00	5·047	100·0	0·0

(From Lusk.)

Table 7

Age.	Calories per Square Metre per Hour.	
	Males.	Females.
14–16 . . .	44·2	41·2
16–18 . . .	41·2	38·2
18–20 . . .	39·2	36·2
20–30 . . .	37·7	35·2
30–40 . . .	37·7	34·7
40–50 . . .	36·7	34·2
50–60 . . .	35·7	33·2
60–70 . . .	34·7	32·2
70–80 . . .	33·7	31·2

(Modified, from Sanborn. "Basal Metabolism," 1922. Sanborn Co., Boston.)

REFERENCES

1. Du Bois, Eugene F.
   Papers in *Journ. Biol. Chem.*, 1910 onwards.
2. Douglas.
   Journ. Physiol., 1911, xlii., xvii.
3. Haldane.
   "*Methods of Air Analysis*," 1918, 137. Griffin & Co., London.
4. Emmes and Riche.
   Amer. Journ. Physiol., 1910, 1911, xxvii., 406–413.
5. Magnus-Levy.
   Von Noorden's "*Metabolism and Practical Medicine*," 1907, i. Keene & Co., Chicago.
6. Rubner.
   Quoted in Sanborn, see below.
7. Frazier and Adler.
   Amer. Journ. Med. Sci., 1921, clxii., 10.
8. Berry.
   Proc. Roy. Soc. Med., 1920–21, xiv., Clin. Sect. and Sect. of Med. and Surg., 4.
9. Aub and Means.
   Arch. Int. Med., 1921, xxviii., 173.
10. Read.
    Journ. Amer. Med. Assocn., 1922, lxxviii., 1887.
11. Harris.
    Brit. Med. Journ., 1923, i., 630.

12. Davies and Eason.
Quart. Journ. Med., 1924, xviii., 259.
13. Du Bois, Eugene F.
Journ. Amer. Med. Assocn., 1921, lxxvii., 352–357.

GENERAL REFERENCES

Macleod.
"*Physiology and Biochemistry in Modern Medicine*," 1924, 547. Kimpton, London.
Dreyer.
Lancet, 1920, ii., 289, 291.
Boothby and Sandeford.
"*Basal Metabolic Rate Determinations*," 1920, 117. W. B. Saunders & Co., Philadelphia.
Sanborn.
"*Basal Metabolism*," by various authors, 1922. Sanborn Co., Boston.

CHAPTER VIII

THE HEART

Cardiac Irregularities

Potain (1), in 1867, obtained tracings from the jugular vein in man. He used as a receiver of its pulsations a glass funnel which was connected by a rubber tube with a Marey sphygmograph, and recorded at the same time tracings from the apex beat, carotid and radial arteries. In 1892 Sir James Mackenzie (2), using a small metal cup as a receiver, devised the instrument originally called a phlebograph, by means of which simultaneous tracings of the pulsations of the jugular vein and radial artery were obtained. The records were taken upon smoked paper, but by the use of inked pens the present-day polygraph has been evolved.

Our knowledge of the essential nature of the different forms of cardiac irregularity is mainly due to Sir James Mackenzie's pioneer work with this instrument, by means of which he obtained records representing the activities both of the auricle and of the ventricle. Further light has been thrown upon this subject and upon myocardial disorders by the use of the electrocardiograph.

The chief varieties of cardiac irregularity are as follows : (1) Sinus arrhythmia ; (2) premature systoles ; (3) heart-block ; (4) auricular flutter ; (5) auricular fibrillation ; (6) paroxysmal tachycardia ; and (7) pulsus alternans.

The Polygraph

This is an instrument for obtaining simultaneous records of pressure changes in the jugular vein and the brachial or

radial artery, and so indirectly of the contraction of the right auricle and left ventricle. There are three main varieties of instrument: (1) The original ink polygraph of Mackenzie; (2) the Mackenzie-Lewis modification; (3) the Jacquet polygraph. The principle is the same in each. A *receiver*, or hollow metal cup, placed over the internal jugular vein, transmits the pressure changes along a rubber tube to the writing tambour which is connected with a pen. The altera-

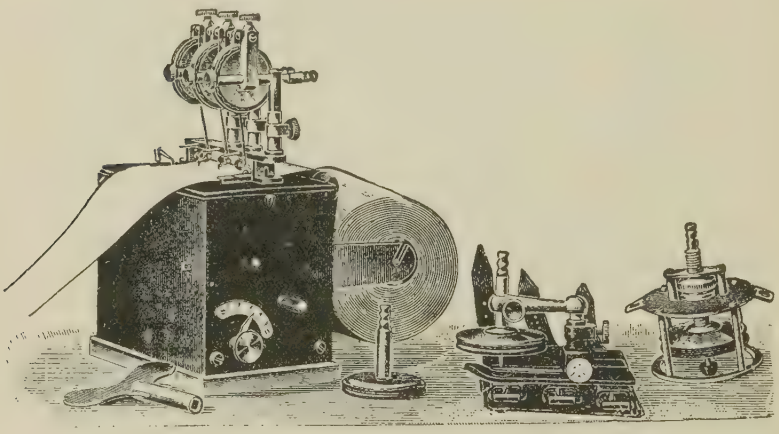


Fig. 8.—The Jacquet Polygraph.

tions in pressure changes in the radial or brachial artery are communicated to a tambour or glycerine pelote, held in position by a suitable armlet. These are transformed by means of another writing tambour into movements of the pen. The inked pens record upon a strip of paper which is mechanically propelled by clockwork at a rate which can be varied at will. Time markings in fifths of a second are also shown by a small pen.

An illustration of the *Jacquet polygraph* appears above.

In this instrument there are three writing pens which allow of the simultaneous record of tracings from the jugular

vein, cardiac apex beat and radial artery. The respiratory movements can be recorded in place of one of the preceding tracings. The small metal pens are always in position for writing and do not require adjustment each time the polygraph is used, as is the case with the Mackenzie and Mackenzie-Lewis instruments. Coloured ink is placed in the groove of the pens, and care must be taken that the ink is not in such amount as to run up to the joint of the pen, as this may cause subsequent stiffness.

The Use of the Instrument

The patient should be lying down comfortably, with all the muscles relaxed and the head supported on a pillow. The metal splint is adjusted so that the pad of the metal spring rests directly over the radial artery, and the wrist tambour is placed in position over it. The pressure is altered by a screw until a satisfactory movement of the corresponding pen is obtained. The venous receiver is then placed in position over the right internal jugular vein, at the level of the jugular bulb, the neck being slightly flexed and turned to the left. The receiver should be placed just above the clavicle and about one inch external to the sterno-clavicular joint. It is held lightly in close apposition to the skin. When oscillations of an appropriate size are obtained, the clockwork is set in action, and the tracing taken. The most favourable spot for a venous tracing varies in different individuals, especially if the sternomastoid be rigid. When a sufficient length of tracing has been procured, the clockwork is stopped and corresponding points upon the venous and arterial tracings marked by moving the pens with the finger. It is important that these ordinates should be marked at the beginning and end of the tracing, or at a space of every twelve inches, to ensure suitable lengths for measuring.

Interpretation of the Tracing

The venous or jugulo-carotid curve shows normally three main waves in each cycle which are termed the *a*, *c* and *v* waves. The interpretation of the underlying causes of their production is open to discussion. The most generally accepted theory is that the *a* wave corresponds with auricular systole, the *c* wave with early ventricular systole, being transmitted from the neighbouring carotid. The *v* wave corresponds with the last phases of ventricular systole just before the opening of the auriculo-ventricular valves, the

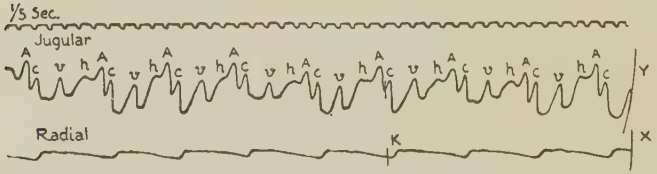


Fig. 9.—Normal Rhythm.

summit of the wave occurring at the time of their opening. The waves on the venous curve are identified as follows :—

The c Wave.—With a pair of dividers measure back from the fixed point (ordinate) *x* on the radial curve to the commencement of any radial upstroke such as *k* (see Fig. 9). Place the dividers, extended to this distance, on the time marking and open them further to the distance occupied by one half of one of the divisions of the time marker, *i.e.*, 1/10 second. This addition is included to allow for the time taken for the arterial wave to pass from the aorta to the wrist. With the dividers adjusted to this distance, place one point on the fixed point (ordinate) *y* on the venous curve, and the other point directed backwards along the venous curve will fall on the commencement of a *c* wave. Other *c* waves on the curve can be identified by taking the distances on the radial curve from the fixed point *x* to other radial up-

strokes, and again adding the distance occupied by 1/10 second, and plotting off this length upon the venous curve.

The v Wave.—One point of the dividers is placed upon the ordinate *x*, and the distance measured back to the bottom of any dicrotic notch on the radial tracing. This distance transferred to the venous tracing, with one point of the dividers placed on the ordinate and the other directed backward, will fall on the summit of a *v* wave. The summit of the *v* wave occurs approximately 2/5 second after the beginning of the *c* wave and has a constant relation to it. Other *v* waves can be similarly identified.

The a Wave.—This is the wave immediately preceding the *c* wave, and occurring in ventricular diastole.

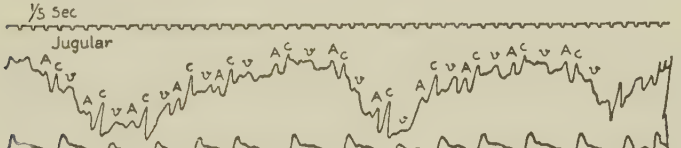


FIG. 10.—Sinus Arrhythmia, showing Relationship of Irregularity to Respiration.

In addition to these three waves, in some normal venous tracings when the heart is beating slowly, a fourth wave may occur. It is known as the *b* wave or the *h* wave, and is seen in diastole usually just before the *a* wave (see Fig. 9). It corresponds in time with the occurrence of a third heart sound, and is thought to be related to a sudden closure of the auriculo-ventricular valves.

In the normal tracing the *a*, *c* and *v* waves are all present, the *a* waves recur at regular intervals, and the *a*–*c* interval is not more than 1/5 second (see Fig. 9).

In the cardiac irregularities variations from the typical tracings occur which are illustrated in the following examples :—

Sinus Arrhythmia.—The irregularity is due solely to

alterations in the length of diastole, which may show a close correspondence with respiration, shortening during inspiration and lengthening during expiration. In the venous tracing the only variations, therefore, are in the length of the *v–a* intervals (see Fig. 10).

Premature Systoles.—These are of three main varieties, auricular, nodal and ventricular, of which the latter constitute about 70%.

Auricular.—The premature auricular systole, marked *a'* in the venous curve, occurs earlier than normal. It may be fused with the preceding *v* wave, and is then of greater amplitude than the ordinary *a* wave. The premature *a* wave is followed by premature *c* and *v* waves, marked *c'* and *v'*.

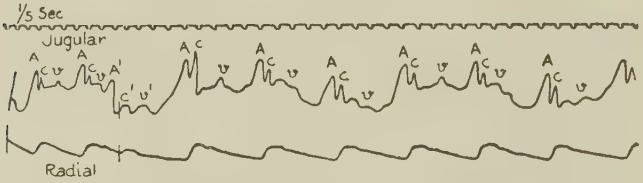


Fig. 11.—Auricular Premature Systole.

The pause in the radial tracing following the premature auricular systole is not quite compensatory, the next beat occurring earlier than it is due in the normal rhythm (see Fig. 11).

Nodal.—The stimulus for the premature contraction arises in the *a–v* node or junctional tissue, and the auricle and ventricle contract simultaneously. The *a* and *c* waves are therefore fused, but the resulting large wave *ca* occurs earlier in the cardiac cycle than a normal *a* wave would do.

The pause in the radial tracing following the premature beat is not usually fully compensatory (see Fig. 12).

Ventricular.—Ventricular premature contractions occur most frequently simultaneously with the normal auricular contraction ; they may take place just before, or just after auricular systole, but in the latter case earlier than normal.

The pause following the premature beat is fully compensatory.

A tracing showing the first variety of premature ventricular systole is shown, the premature contraction *c'* is fused with the auricular contraction, but the large combined *c' a*

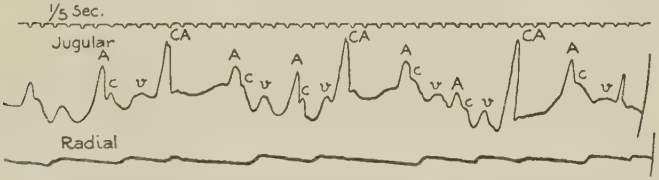


FIG. 12.—Nodal Premature Systoles. Jugular tracing shows large waves due to simultaneous premature contraction of auricle and ventricle.

wave resulting occurs at the moment that an *a* wave is due, and differs therein from the combined *ca* waves occurring in nodal premature systoles (see Fig. 13).

Heart-block.—In the early grades of this condition there is delay in the conduction of the excitatory wave from the

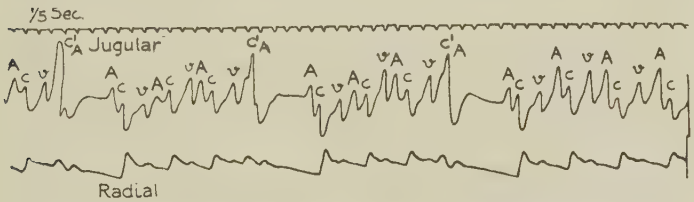


FIG. 13.—Ventricular Premature Systoles. Ventricular premature contractions occur at the same time as the normal auricular contractions, giving a single large wave (*c'a*).

auricle to the ventricle, *i.e.*, in the venous tracing the *a–c* interval is more than 1/5 second (see Fig. 14). The next grade is shown by the occasional failure of the ventricle to respond to the auricular stimulus, in which case the *c* and *v* waves do not appear from time to time, the *a–c* interval usually increasing up to the time of the dropped beat. The

third grade is that in which the ventricular beats are absent in a regular sequence, either every fourth, third or second contraction being missing (see Fig. 15). The terminal stage is complete dissociation between the auricle and the ventricle, the latter beating at its own independent rhythm of about 30 contractions a minute. In this case in the venous tracing

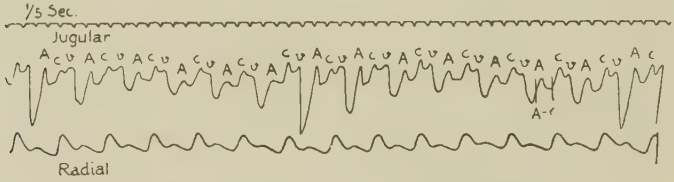


Fig. 14.—Heart-block. Grade I. *a–c* interval exceeds $\frac{1}{5}$ second.

a waves occur at regular intervals, but they may precede the *c* waves, coincide with them, or follow after them (see Fig. 16).

Auricular Flutter.—In flutter the auricle contracts rapidly and regularly, the wave probably pursuing an abnormal circular course. There is usually heart-block, the ventricle

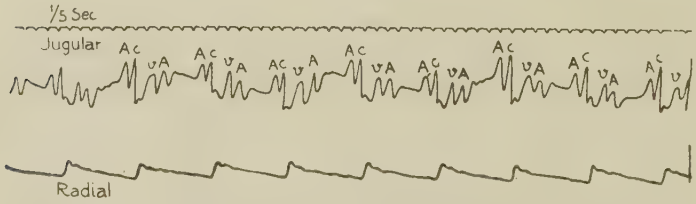


Fig. 15.—2 – 1 Heart-block. Auricular rate = 92 per minute. Ventricular rate = 46 per minute.

responding to every other auricular contraction, or any other degree of block may be present. The ventricular rate is about 160, and may be regular or irregular. The auricular rate is regular and about 200 to 350. The onset and cessation are sudden, but the condition may last for an indefinite period.

The venous curves in auricular flutter are variable, and it may be impossible to recognise the condition by the poly-

graph. The auricular contractions may be too feeble to cause a wave, or the *a* waves may be seen separately, or fused

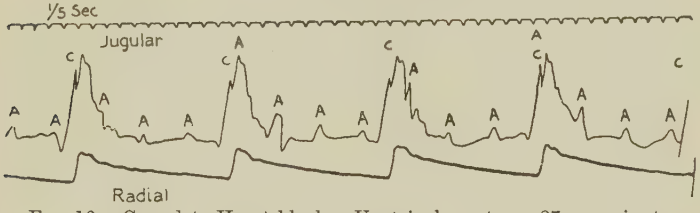


FIG. 16.—Complete Heart-block. Ventricular rate = 27 per minute. The pulse is regular. Auricular rate = 100 per minute.

with *c* or *v* waves. The venous tracing may therefore show a succession of *a* waves, most marked in diastole when the

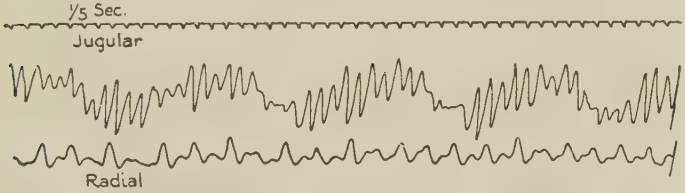


FIG. 17.—Auricular Flutter. Jugular shows regular series of *a* waves, rate 350 per minute. Radial is completely irregular.

ventricle is beating slowly, and larger waves due to fusion with *c* and *v* waves may occur (see Fig. 17).

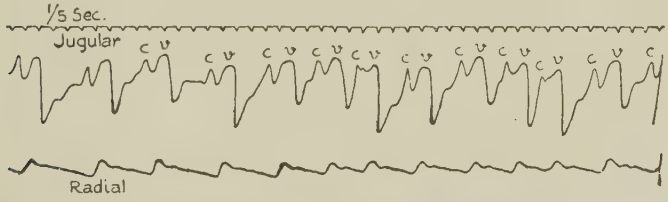


FIG. 18.—Auricular Fibrillation. Jugular shows ventricular type of venous pulse. No *a* waves are present. The pulse is completely irregular.

Auricular Fibrillation.—Here there is no true auricular systole, as the auricle remains in a state of diastole with flickering muscular contractions. The ventricular response

to the auricular stimuli is irregular, both in rhythm and in intensity, but the contractions usually occur more frequently than in a normally beating heart. In the venous curve the *a* waves are absent, or are represented by a few flickers during diastole, and the *c* and *v* waves are quite irregular in time and force. This constitutes the ventricular type of venous pulse (see Fig. 18).

Paroxysmal Tachycardia.—The stimulus for the cardiac contraction occurs at an abnormal site, in the auricle, the A–V node, or the ventricle.

Simple Paroxysmal Tachycardia (*Auricular*).—The pulse rate is usually between 140 and 180, and is not affected by exertion or by lying down. The tachycardia starts and ceases abruptly. The impulse arises at a new focus in the auricle. The *a* wave is often fused with the preceding *v* wave.

Paroxysms of Flutter or Fibrillation.

Nodal Tachycardia.—The auricles and ventricles contract simultaneously. The *a–c* interval is less than 1/5 second, or the *a* and *c* waves are fused.

Ventricular Tachycardia.—A rare condition due to a series of ectopic ventricular systoles.

Pulsus Alternans.—The ventricular rhythm is regular, but alternate contractions are large and small. The radial pulse rhythm is usually not quite regular, the pause following the large beats being slightly greater than the pause after the small beats. The waves usually occur in normal sequence in the venous tracing.

Pulsus alternans may also occur in auricular flutter, or with a series of premature systoles. The regularity of the ventricular rhythm distinguishes the condition from pulsus bigeminus, due to a regular series of premature systoles.

When occurring apart from paroxysmal tachycardia, *i.e.*, in pulses of normal rates, it is an indication of deficient muscular contractility, and is of grave significance.

The Electrocardiograph

The electrical currents produced by the contraction of the human heart were first recorded by Waller (3) in 1887 by means of the capillary electrometer. With this instrument the movements of a fine column of mercury are photographed on a moving plate.

The string galvanometer was later introduced by Einthoven (4) in 1903, and modifications of it are now known as the electrocardiograph (see Fig. 19). The electrocardiograph consists essentially of a thin silvered quartz fibre (GG) running between the poles of a powerful electromagnet (NS) placed very closely together. The fibre is connected by two leads with the body surface (RA and LL). When the heart contracts a current is generated which spreads to the skin, and is picked up by the non-polarisable electrodes attached to the leads. This current causes a minute deviation of the galvanometer fibre, whose shadow is magnified and photographed upon a moving plate. The amplitude of the deflection of the fibre thus produced can be varied by altering its tension, and Einthoven's standard is usually adopted in order that comparable results may be obtained. With a magnification of 450 diameters a difference of potential of one millivolt should cause a deflection of 1 cm. of the fibre shadow.

In addition to the standardiser, which is used as described to correct the fibre tension, the electrocardiograph contains also a compensator (C), by means of which a current can be introduced into the circuit to neutralise currents arising from the skin. A switch connects the galvanometric fibre with any two of the three electrodes which are in contact with the two hands and left foot of the patient.

Simple electrodes, consisting of a zinc cylinder covered with a flannel bag soaked in warm saline, are convenient. The hands, or hand and foot, are inserted into these, the bags being kept in position by tapes, and the saline is pre-

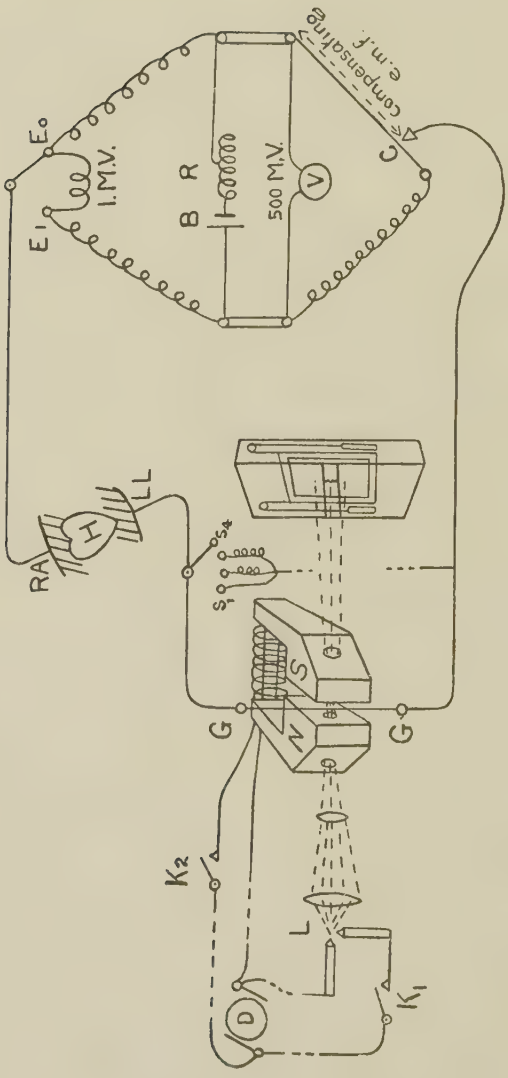


FIG. 19.—Diagram of the Circuit of the Electrocardiograph. (D. T. Harris.)
D = source of current.
S_1 to S_4 = shunt for short circuiting varying proportions of current in galvanometer circuit.
By moving key E_0 to E_1 one millivolt is inserted in the circuit.
B = battery.
R = resistance in compensator circuit.

vented from evaporation by means of a rubber sheet which is wrapped around.

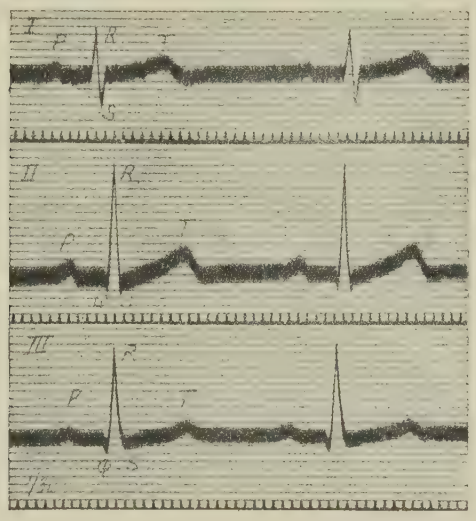
The leads usually adopted are as follows :—

Lead I (transverse). Right and left hands.

Lead II (axial). Right hand and left foot.

Lead III (left lateral). Left hand and left foot.

Curves representing the changes in potential caused by



[*Lewis' "Clinical Electrocardiography."*

FIG. 20.—Normal Electrocardiogram.

the heart's beat are photographed, one below the other, on the same plate. A single beat from the normal heart shows the following waves (see Fig. 20) :—

P is due to auricular systole, and constitutes the auricular complex. **QRST** occur during ventricular systole, and constitute the ventricular complex. **Q** and **S** are often absent. **T–P** represents diastole.

Certain time relations are important :—

The **P–R** interval is normally 0·14 second. Prolongation

of this indicates delay in conduction in the A–V node or the upper part of the bundle of His.

The **QRS** interval is normally just under 0·10 second. Prolongation of this occurs in the various grades of heart-block and in ectopic ventricular beats.

The **R–T** interval is normally 0·32 second. Prolongation of this occurs especially in complete A–V block.

The **QRST** curves are produced by currents originating in the two ventricles, and represent the algebraic sum of the waves produced by the two ventricles contracting separately. By division of one or the other of the two main branches (right or left) of the A–V bundle, the curves produced by the contraction of each ventricle beating separately can be studied, and it is then found that in lead I, **Q** originates in the left ventricle, **R** chiefly in the left ventricle, whereas in lead III, **Q** originates in the right ventricle, **R** chiefly in the right ventricle, and **S** in the left ventricle.

The clinical importance lies in the investigation of bundle-branch lesions which are referred to later.

The characteristic appearance of the deflections in the three leads should now be studied (see Fig. 20). The **R** wave is normally greatest in lead II, and $\mathbf{R}_1$ and $\mathbf{R}_3$ are together approximately equal to $\mathbf{R}_2$. (The figures below the waves indicate the leads in which the waves occur.) The **P** and **T** waves are also usually larger in lead II than in leads I or III.

The Significance of certain Alterations in the Form of the Deflections.—*The P Wave.*—This may be inverted, indicating that the impulse originates at a new focus in the auricle (at times in the A–V node as in paroxysmal tachycardia and in nodal extrasystoles). The **P** wave may be absent, or replaced by a small series of oscillations of a fine or coarse variety, as is seen in auricular fibrillation. It may be abnormally large and notched, indicating auricular hypertrophy, as may occur in mitral stenosis. $\mathbf{P}_3$ may be greater than $\mathbf{P}_2$, as may be seen in auricular flutter.

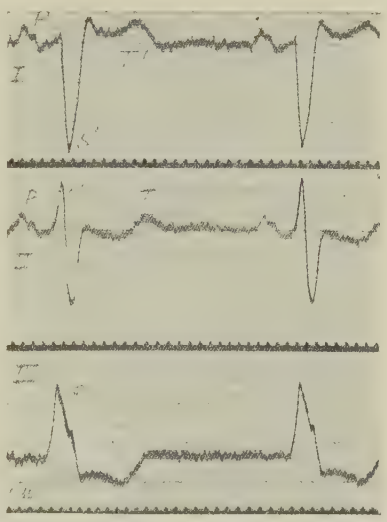
The R Wave.—A maximum **R** wave in lead I indicates

left-sided ventricular preponderance. In right-sided hypertrophy, the **R** wave is greatest in lead III.

Normally the left ventricle is 1·8 times heavier than the right.

The S Wave.—A maximum **S** wave in lead I indicates right-sided ventricular preponderance, whereas a maximum **S** wave in lead III is a sign of left-sided ventricular hypertrophy.

The T Wave.—According to Cowan and Ritchie (5) a positive $\mathbf{T_1}$ wave indicates that at the end of ventricular systole the right ventricle is preponderant over the left, whereas a negative $\mathbf{T_1}$ wave implies the reverse condition. This view is not universally accepted, and by some it is held that the $\mathbf{T_2}$ wave is of great importance as indicating the condition of the ventricular muscle. A negative $\mathbf{T_2}$ wave is always pathological and a negative $\mathbf{T_1}$ wave frequently indicates a pathological condition.



[*Lewis' "Clinical Electrocardiography."*

FIG. 21.—Defective Conduction in Left Branch of the Bundle.

Prolongation of the QRS Complex.—In the condition known as bundle-branch block, the right or left branch of the bundle of His is impaired and fails to conduct. The **QRS** complex is prolonged or spread out, and lasts longer than the normal maximum of 0·10 second. The **R** wave may be notched. The **T** wave is in the opposite direction to the main initial deflection (**R** or **S**), and is usually of a greater ampli-

tude than normal. The ventricular complex is therefore diphasic.

Left Branch Bundle Block.—The main initial deflection ($\mathbf{S}_1$) is negative in lead I and positive ($\mathbf{R}_1$) in lead III (see Fig. 21).

Right Branch Bundle Block.—The main initial deflection ($\mathbf{R}_1$) is positive in lead I and negative ($\mathbf{S}_1$) in lead III (see Fig. 22).

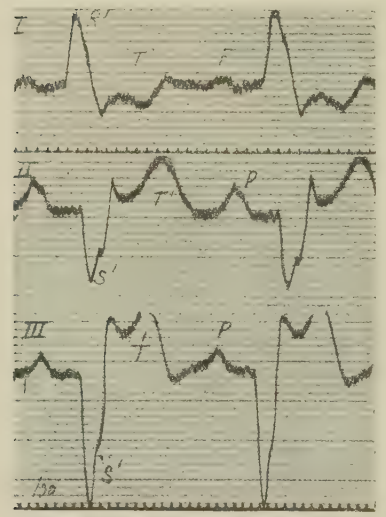


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FIG. 22.—Defective Conduction in Right Branch of the Bundle.

The **QRS** complex is also prolonged in *intraventricular block*, in which the terminations of the bundles in the ventricular subendothelial tissue are destroyed. The **R** wave is also often notched.

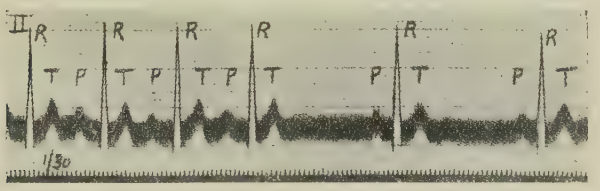
In *congenital dextrocardia* all the waves in lead I are inverted, whereas lead II may represent the normal lead III, and *vice versâ*.

Absence of the PRT Complex.—This occurs in sino-auricular block, in which the stimulus arising in the S–A node at times fails to cause a contraction of the auricle. The whole heart, therefore, misses a beat, but the succeeding contraction occurs at approximately the normal interval. This is not necessarily a serious condition (see Fig. 23).

In addition to the light which the electrocardiograph throws upon the condition of the myocardium and conducting tissue, the various types of cardiac irregularity previously described in the section dealing with the polygraph can be accurately determined.

Sinus Arrhythmia.—Variations in the length of the **T–P** intervals are seen, but the auricular and ventricular complexes are normal.

Premature Systoles.—*Auricular.*—The premature impulse may arise at the S–A node, near it, or some distance away.



[*Lewis' "Clinical Electrocardiography."*

Fig. 23.—Sino-auricular Block.

In the two former instances the **P** wave caused by the premature contraction is in the normal direction, in the latter it is inverted, indicating that the impulse spreads through the auricle along abnormal paths (see Fig. 24). The ventricular complex following the premature auricular stimulus is

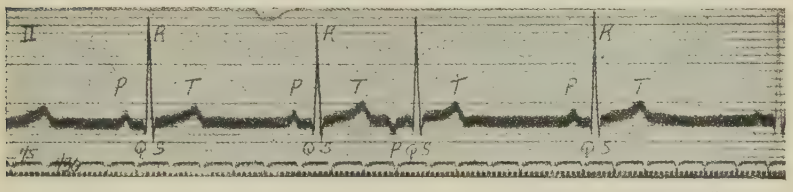


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Fig. 24.—Premature Auricular Systole.

usually of normal type. The premature **P** wave may occur so early in diastole that it fuses with the previous **T** wave.

Usually the diastole following the premature beat is not fully compensatory.

Nodal.—The premature impulse arises in the junctional tissue uniting the auricle and ventricle, *i.e.*, in the A–V node or the A–V bundle before division. The ventricular contrac-

tion may occur just before the auricular, but is seen to be supra-ventricular in origin because the **QRS** complex is of normal appearance (see Fig. 25).

Ventricular.—A premature stimulus arises in the ventricle

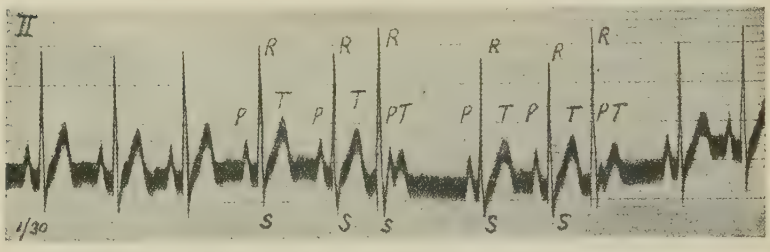

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FIG. 25.—Premature Nodal Systoles.

below the division of the main A–V bundle. The premature ventricular complex resulting has the same duration as a normal ventricular complex, but its form is altered, the initial deflections of the premature beat being larger than normal. The auricular waves occur at regular intervals, and may be fused with the premature ventricular deflections, or appear just after them. The premature ventricular beat is followed by a prolonged diastole and the succeeding pause is thus fully compensatory.

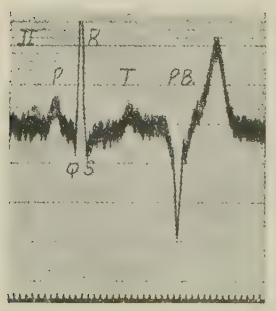

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FIG. 26.—Premature Systole arising in the Left Ventricle.

Left Ventricular Premature Contractions.—Lead I. The premature contraction is shown by an upward, followed by a downward deflection.

Leads II *and* III. The premature beat is shown by a downward, followed by a smaller upward deflection (see Fig. 26).

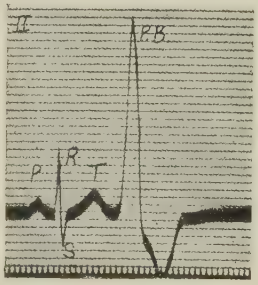
Right Ventricular Premature Contractions.—The appear-

ance in the leads is the reverse of that which is seen in premature contractions originating in the left ventricle (see Fig. 27).

Heart-Block.—Various degrees of impairment of conductivity of the heart muscle can be distinguished by the electrocardiograph.

Slight.—Prolongation of the **P–R** interval to more than 0·2 second.

Occasional Dropped Beats.—The **P–R** interval gradually becomes longer until, finally, the ventricular complex fails to follow its preceding auricular complex. The **P** waves are quite regular, but the ventricular complexes occur at different intervals, and the **P** and **T** waves may at times fuse.



[*Lewis' "Clinical Electrocardiography."*

FIG. 27.—Premature Systole arising in the Right Ventricle.

Regular Dropped Beats.—Every fourth, third or second ventricular contraction may be omitted, constituting a 4 : 1, 3 : 1, or 2 : 1 heart-block.

Complete Heart-block.—The ventricle contracts at its own independent rate of about 30 beats a minute. The **P** waves occur regularly, and may be seen isolated on the electrocardiogram or fused with **R** or **T** waves. The ventricular complex is of normal duration and configuration, and so the impulses giving rise to it are supra-ventricular, but as they do not originate in the auricle, they must be derived from the junctional tissue (see Fig. 25).

Bundle-branch Block and *Intra-ventricular Block* have already been described (p. 252) (see Figs. 21, 22).

Sino-auricular Block (*Tortoise Heart*).—Some of the impulses arising in the S–A node fail to reach the main mass of auricular muscle, and occasional intermittences of the

whole heart may occur. If the rate be slow, as is usually the case, on exercise it may increase to about double (see Fig. 23).

When the heart is beating at such a slow rate the ventricle may interpolate a beat on its own (*ventricular escape*), the

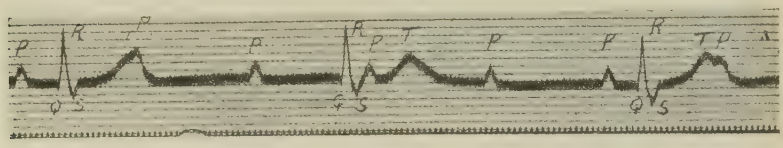


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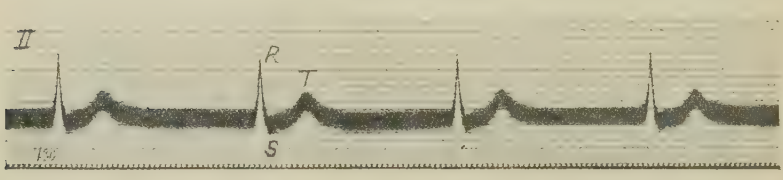
Fig. 28.—Complete Heart-block (Lead II).

P–R interval being shortened, or the **P** and **R** waves may partially fuse.

Nodal Rhythm.—The impulse may arise at the A–V node instead of at the S–A node, the auricles and ventricles contract simultaneously, and no **P** waves are seen (see Fig. 29).



[*Lewis' "Clinical Electrocardiography."*

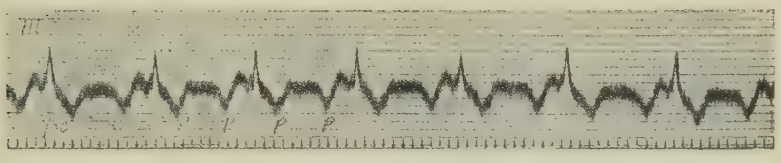
Fig. 29.—Nodal Rhythm.

Auricular Flutter.—An electrocardiogram shows the auricular contractions occurring at a regular rate, with ventricular complexes superimposed upon the curve at regular or irregular intervals. Heart-block of varying grades is almost invariably present.

The **P** waves, which occur at regular intervals, and have a dome-shaped appearance, must not be mistaken for inverted **P** and **T** waves. In some tracings the **T** wave is also apparent (see Fig. 30).

Pressure on the vagus in the neck may slow the rate of ventricular contraction, but the **P** waves continue to come through at regular and rapid intervals.

The auricular contraction is believed to pursue an abnormal circular path in the auricle. When the normal rhythm is established, as the result of the administration of digitalis

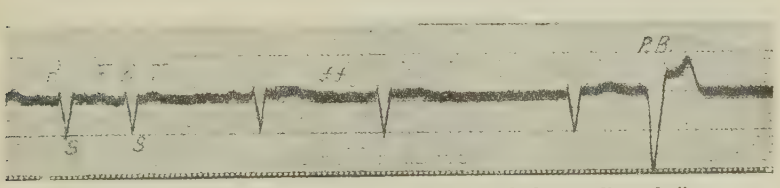


[*Lewis' "Clinical Electrocardiography."*

FIG. 30.—Auricular Flutter. Auricular Rate = 324. Ventricular Rate = 162.

or of quinidine, the gentle undulations of the **P** waves of flutter are replaced by a sharper peaked wave of normal rhythm.

Auricular Fibrillation.—Here the **P** waves are absent, and **QRS** deflections occur at irregular intervals, and are of varying height. The ventricular complex is of normal form.



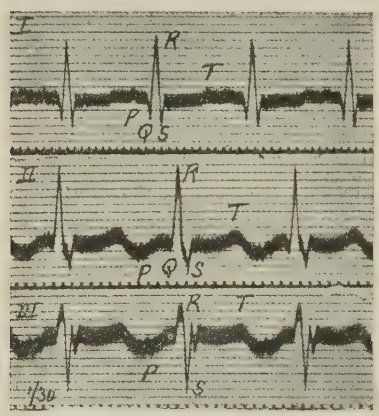
[*Lewis' "Clinical Electrocardiography."*

FIG. 31.—Auricular Fibrillation Showing a Single Ectopic Ventricular Beat (Lead III).

Oscillations of irregular size may be seen in diastole, caused by the fibrillating auricle ; these may be fine or coarse. They are usually best seen in leads II and III. They also occur during systole, and may alter the shape of the **T** wave, although the **QRS** deflections are not affected. In rapidly beating hearts the oscillations are usually not well seen in diastole, but their effect upon the **T** wave is generally noticeable.

The **QRS** deflections may occasionally be seen at regular intervals, although the auricle is fibrillating and no **P** waves are present, but oscillations due to fibrillation may be seen in diastole. This occurs when the ventricle has adopted its independent rhythm owing to complete heart-block.

Ectopic beats may arise during fibrillation; they are ventricular in origin. They may occur regularly after each **QRS** deflection, due to a supraventricular stimulus, constituting the coupled beats. This is usually due to excessive digitalis administration, and is a sign for immediate discontinuance of the drug (see Fig. 31).



[*Lewis' "Clinical Electrocardiography."*

Fig. 32.—Simple Paroxysmal Tachycardia, showing Inversion of the P. Wave in Leads II and III.

Ventricular Fibrillation.—Although compatible with life in man, if only of short duration (Gallavardin and Bérard (6)), it is the probable cause of sudden death from heart failure, as occurs under anæsthetics, etc.

Paroxysmal Tachycardia.—*Simple Paroxysmal Tachycardia* (*Auricular*).—A succession of ectopic auricular contractions occurs, arising at a new focus in the auricle. This is shown in the electrocardiogram by a rapid regular rhythm of about 150, in which the ventricular complexes are normal. The impulse has, therefore, a supraventricular origin. The **P** wave is often inverted in leads II and III, and usually modified in lead I. The auricular impulse is therefore ectopic in origin, and if not inverted in leads II and III it is usually smaller than normal (see Fig. 32).

When the paroxysm ceases there is a pause, which is

immediately followed by the restoration of the normal rhythm.

Auricular Flutter (see p. 256).

Auricular Fibrillation (see p. 257).

Nodal, arising in the A–V node. Here the auricle may contract first and the **P–R** interval be shortened, and **P** inverted, or the auricle and ventricle may contract simultaneously and the **P** and **R** be fused.

Ventricular.—A rare condition characterised by a succession of ectopic ventricular systoles.

Pulsus Alternans.—This condition, which is revealed by a tracing from the arterial pulse, is not always shown by the electrocardiograph. Thus, the **R** waves may be of equal intensity although the pulse shows marked alternation. At times the **R** waves may show alternation, but the large **R** waves may correspond with the small pulse waves, and *vice versâ*. Occasionally the electrocardiogram shows alternation of the **R** waves, although the pulse tracing is regular.

The Practical Value of Graphic Methods in Affections of the Heart.—Having considered the common variations which may be met with in polygraphic and electrocardiographic tracings, the question arises as to what is their actual value in practice, and when should these methods be employed. The polygraph is an instrument which is comparatively inexpensive, is portable, not likely to get out of order, and whose use can be learnt in a short time. It yields, however, very little information as to the state of the heart muscle. Long strips of tracing can be taken with the polygraph, which are more likely to show an irregularity which occurs infrequently than is an electrocardiogram in which only a few beats are recorded. The electrocardiograph possesses qualities which are the reverse of those described as belonging to the polygraph, but, on the other hand, it is the only way of investigating many varieties of cardiac irregularity and of determining the functioning power of the cardiac muscle. Broadly speaking, all valvular lesions are diagnosed

without instrumental aid by the ordinary methods of clinical examination. Of the irregularities, sinus arrhythmia, premature systoles, auricular fibrillation, and some grades of heart-block, can usually be diagnosed without instruments.

The polygraph will show pulsus alternans, will determine the site of origin of the impulse in premature systoles, and the varieties of supraventricular heart-block. It will distinguish a slow fibrillation from premature systoles and may throw light upon cases of auricular flutter and paroxysmal tachycardia.

The electrocardiograph is of special value in determining the condition of the heart muscle, the relative preponderance of ventricular activity, the state of the conducting paths after their entry into the ventricles, and the varieties of paroxysmal tachycardia and flutter. It is also diagnostic of congenital dextro-cardia. The question of the value of an electrocardiogram in cases of Graves' disease may arise, and Goodall and Rogers (7) have shown that in acute thyrotoxicosis the electrocardiogram differs from that obtained in a case of physiological tachycardia. In the former the **P** waves reach the level of the **T** waves, and the **P–R** interval is often prolonged. In the latter the **P** waves are always smaller than the **T** waves. This is probably due to a hyaline type of myocardial degeneration.

The Determination of Blood Pressures

It is now usually accepted that attempts to estimate the blood pressure by means of the tactile sense alone, without instrumental aid, are misleading and unreliable. There are, however, certain authorities who maintain that this is not the case. Thus William Russell (8), writing in 1921, states: "The finger is still the main means of estimating blood pressure in clinical work." This view is based on the belief that considerable pressure may be required to obliterate an artery thickened by degenerative changes, or in a state of hyper-

tonus. It is usually thought that the force thus expended in blood pressure determinations is comparatively negligible, but Russell, from experimental observations, concludes that in some instances a pressure of 150 mm. Hg or more may be required to collapse a thickened artery. In such a case a high pressure, as recorded by an instrument, would not connote a high intra-arterial tension.

The work of MacWilliam and Kesson (9) is opposed to Russell's views. They showed that, at body temperatures, only a moderate degree of arterial hypertonus can exist. Further, such spasm as is present disappears with massage of the arm, or as the result of the pressure of the sphygmomanometer armlet if it be kept inflated for a few minutes. This corresponds with the fall in systolic pressure, which is often observed, if successive readings are taken within a few minutes of each other, until, finally, what is known as the *residual systolic pressure* is obtained. Further, atheromatous and calcareous changes in the artery appear to have little effect upon the pressure required to compress the vessel, as such changes are usually localised and rarely affect a strip of artery uniformly for a length of 10 or 12 cm., such as is compressed under the armlet. Obliteration of the lumen of the vessel, therefore, occurs at some supple spot under pressure. The increase of pressure, due to changes in the vessel wall, probably does not amount to more than 5 to 10 mm. Hg if care be taken to eliminate arterial spasm by taking several readings until the *residual systolic pressure* is obtained.

It is quite impossible to determine, even approximately, the diastolic pressure without instrumental aid. It is important to investigate it for several reasons. The diastolic pressure may be raised without a corresponding increase in the systolic pressure. This involves a continuously increased strain on the vessels. The prognosis with diastolic pressures which are constantly 135 mm. Hg or over is very grave: The diastolic pressure may be abnormally low, although the

systolic is raised, as is found typically in aortic regurgitation with a free reflux. It is necessary to determine the diastolic pressure in order to calculate the pulse pressure. An increased pulse pressure is associated with hyperthyroidism, as described on p. 227. Another example of the value of instruments in taking blood pressure is the fact that the condition of pulsus alternans may sometimes be diagnosed by this means, as described below, although it is not perceptible to the finger alone.

The chief methods employed for determining the blood pressure may be classified as follows :—

Palpatory.
Vibratory.
Auscultatory.
Oscillatory.

Palpatory.—*Systolic Pressure.*—Von Basch of Vienna (10), in 1876, first devised a clinical method for estimating the systolic blood pressure in man. Previously the blood pressure in animals had been determined by introducing a cannula directly into an artery, as was done by the Rev. S. Hales (11) on a horse in 1773. Faivre (12) also, in 1856, had adopted a similar procedure in man, inserting a cannula attached to a mercury manometer into the arteries of a limb about to be amputated. He recorded a systolic pressure of 120 mm. Hg in the femoral and 115 to 120 mm. Hg in the radial artery.

Von Basch employed a pelote consisting of a small glass funnel connected by a rubber tube with a mercury manometer, the open end of the funnel being closed by an elastic membrane. The pelote and tubing were filled with water. The systolic pressure was determined by compressing an artery, such as the radial or temporal, between the pelote and the underlying bone, and at the same time feeling the pulse beyond the site of the pressure. The force required to obliterate the pulse could then be calculated by means of the manometer.

Potain (13), in 1889, modified this instrument, using a rubber pelote communicating by air transmission with a metal manometer. It was very popular in France, and was used there almost exclusively for the next twenty years. Readings by either of these forms of sphygmomanometers give figures for the systolic pressure which are often 50 or 60 mm. Hg too high, owing to the fact that the artery is compressed on one side only, and that through the medium of the intervening soft tissues.

Riva-Rocci (14), of Pavia, at the Italian Congress of Medicine in 1890, introduced the method of compressing the artery by means of an armlet encircling the limb, into which air is pumped. This armlet had originally a width of only 5 cm.

Von Recklinghausen (15) showed subsequently that the pressures recorded are unduly high unless the armlet has a width of 12 or 13 cm. The method of determining the blood pressure by the Riva-Rocci instrument is well known. It constitutes *the* most accurate means of estimating clinically the systolic blood pressure in man. The results obtained are about 7·5 % higher than the true systolic pressure. If the reading be taken from the brachial instead of from the radial artery, the figures are from 2 to 8 mm. higher still. Care must be taken to determine the *residual systolic pressure* by making several estimations until a constant reading is obtained. Venous stasis caused by too long compression of the arm must also be avoided. Gallavardin (16) advises that the point at which the pulse disappears on compression should be determined rather than its point of reappearance on decompression, but the mean of these two readings is often taken as the systolic pressure. In *pulsus alternans* a difference in systolic pressure of as much as 35 mm. Hg between the alternate large and small beats may be observed (Gallavardin (16)).

Diastolic Pressure.—Strasburger (17), in 1904, described a method for determining the diastolic pressure with the Riva-

Rocci apparatus. The radial pulse is carefully felt while the armlet is slowly inflated, and the point is noticed at which there first occurs a diminution in the force of the beat. This indicates approximately the diastolic pressure, but the figure obtained is a little too high.

Vibratory.—This is a method for determining the diastolic pressure only. Ehret (18), in 1909, drew attention to a phenomenon which Strasburger had also previously noted, without realising its significance. When the pressure is slowly raised, as in Strasburger's method, just before the pulse becomes perceptibly smaller, the artery suddenly pulsates vigorously, giving a strongly vibrating sensation. This is much more clearly appreciated if the brachial rather than the radial artery be palpated. As the pressure is further raised these forcible vibrations first increase and then decrease in intensity for a period of about 5 mm. of the pressure scale, and are then replaced by normal beats which disappear when the systolic pressure is reached. On lowering the pressure the reverse takes place, and the point at which the vibrations disappear on decompression is usually 2 to 3 mm. lower than that at which they are first noted on compression. They are especially well marked in cases of high blood pressure. *The diastolic point is taken as that pressure at which the first normal beat follows a vibrating beat on decompression.* The results obtained are very accurate and correspond closely with the figures given by the auscultatory or oscillatory methods. In about 4 to 10% of all cases it is found to be impossible to estimate the diastolic pressure by this means, and in cases of aortic regurgitation it is often especially difficult. In *pulsus alternans* the diastolic pressure is alternately higher and lower, and if the pressure in the armlet be maintained at a point intermediate between the two diastolic values, the beats felt over the brachial artery will be alternately vibrating and flat in quality.

Auscultatory (*Systolic and Diastolic Pressures*).—Korotkow (19), a Russian physician, introduced in 1905 this simple

and accurate method for determining the blood pressure. Its particular value lies in its affording a very reliable means of estimating the diastolic figure. Two instruments are required, a sphygmomanometer of the Riva-Rocci type, with a mercury or anæroid manometer, and a stethoscope, preferably of the phonendoscope variety. The phonendoscope may be fixed in a band by which it can be strapped in position over the arm, leaving the two hands free. This, however, is not necessary for routine work.

The patient should be lying on a couch or sitting with the arm comfortably resting on a table at the level of the heart. There must be complete silence in the room. The armlet is applied as high up the upper arm as is possible, with the centre of the rubber bag lying over the brachial artery. The position of the artery on the inner side of the biceps tendon should be determined, so that the stethoscope can readily be applied over it. The pressure in the armlet is raised fairly quickly until the pulse at the wrist is obliterated, the stethoscope is then applied over the brachial artery, taking care not to compress the vessel, and the pressure in the armlet is gradually lowered. No sound is audible until the pressure falls to the systolic level, when a series of little taps or thuds are heard as the blood commences to flow through the compressed artery (*phase* 1).

On lowering the pressure still further these initial sounds are replaced by a soft murmur (*phase* 2). With further decompression the murmur disappears, and a series of loud clear sounds, which have been likened to the beats on a gong, are heard (*phase* 3). These increase and decrease in intensity, and, with further fall in pressure, they suddenly change in tone and intensity and become dull and muffled sounds (*phase* 4), which soon disappear completely (*phase* 5). This succession of sounds, which are better heard on decompression than on compression, constitute the five phases of Ettinger (20), and are separated from each other by certain points. *Point* 1 is the moment at which the first sound is

heard on decompression and gives the systolic pressure. It is usually a few millimetres higher than that obtained by the palpatory method. It should always be confirmed thus, and if there be any marked discrepancy the figure obtained by palpation should be accepted as representing the systolic pressure. We have seen above that the figure obtained by palpation is itself higher than the true systolic pressure. The phases are separated from each other by points 2, 3, 4 and 5,

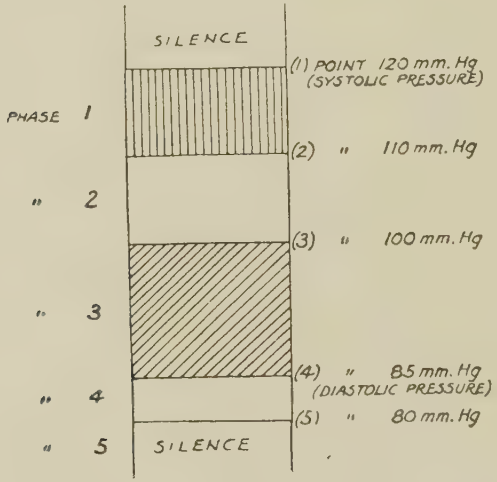


FIG. 33.—The Phases of Normal Blood Pressure.

the latter being placed at the end of phase 4, *i.e.*, when silence commences. *The whole value of the method depends upon the accurate determination of point* 4, *as this represents the diastolic pressure.* It is that point at which the loud clear sounds of phase 3 are suddenly replaced by the dull and muffled sound of phase 4.

The Normal Duration of the Pressure Phases.—These may be represented diagrammatically as above (see Fig. 33) :—

Phase 1. Is always present and normally lasts about 10 mm. Hg.

Phase 2. Is very inconstant. The murmur is not heard in some cases of high blood pressure, a silent gap, or *trou auscultatoire*, taking its place. It may disappear altogether, phase 1 passing directly into phase 3, if the arm be kept compressed tightly for a short time, probably owing to venous stasis. It usually lasts from 5 to 10 mm. Hg.

Phase 3. Occupies a large part of the cycle and is the most obvious sound heard. It persists for about 15 to 20 mm. Hg.

Phase 4. In normal adults is of short duration, about 5 mm. Hg. In young children it is longer, and, according to Tixier (21), it persists for 20 mm. between the age of 20 and 30 years, and for 10 mm. between 30 and 40 years of age. In aortic regurgitation it may be very much prolonged, and sounds may be heard almost to the point at which decompression is complete.

That point 4, *and not point* 5, *accurately represents the diastolic pressure* has been conclusively demonstrated by various workers, especially by MacWilliam and Melvin (22) by experiments upon sheep, and by Warfield (23) on dogs.

This does not appear to be sufficiently appreciated, and from time to time practitioners and students are met who are taking point 5 as being the diastolic index. The whole value of the method is thereby vitiated, for its importance lies in this alone, that it affords a simple and accurate method for determining the diastolic pressure.

In about 10% of cases it is found impossible to decide where point 4 occurs, and this is especially so when aortic regurgitation is present and when the arteries are hard and small.

Oscillatory.—This is a method for determining both systolic and diastolic pressures. It has been extensively employed clinically in France, with the help of Pachon's oscillometer (24), and, in America particularly, Erlanger's sphygmomanometer (25) is often employed.

Marey (26), in 1876, introduced the oscillatory method for determining the diastolic pressure by means of his sphygmoscope.

The Pachon Oscillometer.—This was introduced in 1909; it is a complicated instrument, and its readings are often hard to interpret. The apparatus consists of a rigid metal box, containing a manometric capsule, and a pressure manometer and armlet. The manometric capsule enables the extent of the oscillations to be read at different pressures. It is put into communication with the circuit, consisting of box, manometer and armlet, by means of a screw, and the oscillations read, centimetre by centimetre, as the pressure is lowered. Small oscillations are first seen on decompression, which become larger and then decrease in size. The difficulty in interpreting the readings lies essentially in the fact that often there are oscillations at pressures above the systolic figure and below the diastolic. These are called supramaximal and inframinimal oscillations.

The systolic pressure is that pressure at which the first large oscillations occur on decompression. If there be no supramaximal oscillations present, this is easy to determine; if, however, they occur, the systolic pressure is that point at which the supramaximal oscillations are replaced by large oscillations. The supramaximal oscillations are due to waves of blood beating against the obstruction caused in the brachial artery by the pressure of the armlet.

In any case the systolic pressure as recorded by the Pachon oscillometer is inaccurate, being far too high. There is an error of about 30%, and the normal systolic pressure for an adult works out at about 140 to 150 mm. Hg.

The diastolic pressure.—This also presents difficulties. Pachon originally said that it was the point at which the oscillations are greatest. *It is, however, now taken as that point at which the large oscillations undergo a sudden and marked diminution in their size, and become inframinimal.* The average diastolic pressure for an adult with the Pachon instrument is about 90 mm. Hg.

In about 8% of cases it is impossible to determine the diastolic pressure by the oscillatory method, as there is no

sharp point of demarcation between the two types of waves.

The Erlanger Apparatus.—The oscillations are recorded graphically on a blackened paper travelling with a moving drum.

The systolic and diastolic points are as in the Pachon readings, and present the same difficulties in interpretation.

Variation in Blood Pressures with Age.—The average systolic pressure at birth is from 35 to 55 mm. Hg. Various formulæ have been proposed for calculating the normal blood pressure at different ages, but there is naturally a certain physiological degree of latitude, and the rule put forward by Rolleston (27) is of value. It is as follows : The ideal systolic pressure for an adult is the figure represented by 100 plus half the age in years. The maximal physiological pressure is 100 plus the age. Further, it may be added that a systolic blood pressure higher than 150 or 155 mm. Hg at any age is abnormal. The diastolic pressure is roughly two-thirds the systolic up to middle age, but after this it is frequently only half the systolic.

It will be observed that the pulse pressure, namely, the difference between the systolic and diastolic pressures, normally remains very constant at different ages, between 42 and 49 mm. Hg, and the normal ratio between systolic, diastolic and pulse pressures is as 3 : 2 : 1.

In women the systolic pressure is usually 5 to 10 mm. Hg lower than in men.

Some Cardio-therapeutic Measures

The Use of Digitalis and Strophanthus.—Digitalis still remains supreme amongst the drugs used in the treatment of heart disease. Withering (28), in 1785, was one of the first to draw attention to the value of digitalis in producing diuresis in certain cases of heart failure. Since then the indications for its use have become more clearly defined, and

it is recognised that its most beneficial effects are obtained in cases of cardiac irregularity due to auricular fibrillation. It does not abolish the *circus* movement in the auricle, but slows the ventricle by increasing the degree of heart-block. It is also of value in auricular flutter, and, indeed, in heart failure from any cause. High blood pressure is not now considered a contra-indication, as it has been shown that in therapeutic doses digitalis does not cause a rise of systolic pressure, although the pulse pressure may be increased as the result of a fall in the diastolic level in cases of cardiac failure. Whether or not it acts as a direct *cardiac tonic* and should be employed in disorders associated with sinus rhythm is a matter of dispute. It is usually held that digitalis in such cases slows the whole heart by its action on the vagus, thus prolonging diastole, increasing the force of systole and improving the coronary circulation and so the nutrition of the cardiac muscle.

In heart failure of a severe degree large doses are usually required. On account of the danger of producing severe toxic effects, the total quantity necessary is generally administered in divided doses at several hours' interval, but the beneficial effect is thereby inevitably postponed. This delay is partially due to the fact that digitalis takes about six hours before it is absorbed from the stomach; it is also eliminated slowly by the intestines and kidney, and so cumulative results may occur.

It has been shown experimentally that the maximum beneficial effect is produced by that quantity of digitalis which is just sufficient to cause signs of intoxication, and, further, that that amount is proportional to the body weight of the individual.

In order to obtain results in cases of severe heart failure as quickly as possible, Eggleston (29) has devised a method for determining the maximum amount of the drug which can safely be administered to an individual. Digitalis in America is standardised in *cat units*, whereas in England the frog is

the animal usually employed in its biological assay. The *cat unit* of Hatcher (30) is the weight of dry drug in milligrams per kilogram of body weight which is required to kill a cat when a continuous intravenous injection of the solution is given slowly. This is approximately equivalent to 0·1 g. of a good quality dry leaf, or 1 c.c. of the tincture.

The amount of the tincture required to produce the maximum beneficial effect in man can be determined by Eggleston's formula (31). For the tincture of digitalis it is as follows :—

$$\frac{CU \times 0{\cdot}1 \times W}{100} = \text{c.c. of tincture in total amount,}$$

and for the powdered leaf it is :—

$$\frac{CU \times 0{\cdot}1 \times W}{1{,}000} = \text{g. of powdered leaf in total amount.}$$

Here CU = the number of milligrams of the specimen of dry digitalis which is being used that constitutes one cat unit. W = weight of patient in pounds. If the cat unit value be unknown, it may be taken to be 100 for a good tincture or for the leaf. For a man weighing 10 stones the amount required is 14 c.c., or 4 dr. of tincture, and for a man of 8 stones about 3 dr. When the weight of the patient is not known accurately, as will often be the case, three-quarters of the above amounts should be the maximum given, *i.e.*, 3 dr. for a man of about 10 stones, and $2\frac{1}{4}$ dr. for a man weighing about 8 stones.

The Body Weight Method.—The maximum effect is produced in ten to twenty hours. The total calculated amount is given in the following doses : One-third to one-half of the total as the first dose, one-fifth to one-quarter of the total six hours later, and one-eighth to one-sixth the total after another six hours. If more digitalis be required, one-tenth of the total is given six-hourly until the maximal effect is produced. This method must only be used in very urgent

cases in which no digitalis has been given within the last ten days.

If the case be not very urgent the dose is divided and spaced as follows :—

One-quarter of the total calculated amount for the first dose, repeated after six hours ; then one-tenth to one-eighth of the total six-hourly until the maximum effect is produced.

If digitalis has been given within the last ten days the total calculated amount should be reduced to three-quarters if no signs of digitalis over-action be shown by the polygraph or electrocardiograph, such as heart-block or premature ventricular systoles, and to one-half if they be present. In either case the total calculated amount is given in three equal doses at six-hourly intervals.

After digitalisation has been effected the drug should be discontinued for a day or so. Then 20 m. or less three times a day may be required to allow for the daily elimination, and to keep the patient under the influence of the drug. This may be necessary for long periods.

Cat Unit tablets are now available, prepared from the digitalis leaves and standardised by the pharmacological laboratories of the Pharmaceutical Society of Great Britain. Each tablet contains one cat unit, corresponding approximately with 1 c.c. of the tincture or 0·1 g. of the leaf, and one cat unit is required for every 10 lb. of body weight of the patient (31). Thus for a man weighing 10 stones, the total amount required will be fourteen tablets, in order to produce the full digitalis effect. As about two cat units a day are lost by elimination, an extra two tablets are required each day the drug is exhibited.

Thus if digitalisation is to be effected in one day, sixteen tablets in all would be required, given at intervals of six hours, four tablets in each dose. If two days are to be taken before the full effect is produced, then eighteen tablets in all will be necessary, nine each day, given three at a time, three times a day, at intervals of six hours.

The indications for discontinuing the drug are, according to Eggleston : Nausea or vomiting, unless this is due to venous congestion of the stomach, which is usually the case when it occurs shortly after the commencement of digitalis administration (Jensen (32)). The apex rate lower than 60. The occurrence of frequent ectopic systoles, heart-block, phasic arrhythmia or coupled beats. The latter are caused by a regular sequence of premature ventricular systoles following each normal ventricular contraction.

It is usually found that if the digitalis is going to relieve the heart failure diuresis takes place ; if there be no diuresis, beneficial results are not likely to ensue, and diminution in the secretion of the urine is an additional sign of digitalis toxæmia. The diuresis is a manifestation of the effect of digitalis in filling the arteries and emptying the veins, and is due to an increased renal circulation and relief of renal congestion. Diuresis does not follow the administration of digitalis, even in massive doses, unless œdema is present.

Digitalis should not be administered more frequently than at six-hourly intervals, as this is the time taken for its absorption from the stomach, and in cases of auricular fibrillation it has been shown by Canby Robinson (33) that an effect is produced in two to five hours after oral administration. Sufficient warning of any toxic effects, which may be produced by its administration, is thereby given.

It is clear that, with such massive quantities, there must be a risk of overdosage if one be not certain of the condition of the junctional tissue and of the myocardium, and so, unless the symptoms are very urgent, it is not wise to adopt such measures.

Fraser (34) has tried the method in England in ten cases. Good results were obtained in auricular fibrillation, but in cases with normal rhythm he concludes that the time gained in producing the digitalis effect does not justify the risk of overdosage. In conclusion, it may be said that massive doses should only be used in cases of heart failure of extreme

urgency, and that usually administration in smaller doses is the safest course to adopt.

Nativelle's Granules of Digitalin (gr. 1/240 or 1/600).—This preparation is of value in those patients who show marked gastric intolerance to the other preparations of digitalis. A granule containing 1/240 gr. of digitalin corresponds approximately to 15-20 m. of the tincture. The granules may be tried also in cases which do not respond well to the tincture and form a convenient means of administering the drug in small doses over a prolonged period, as is usually required in the treatment of auricular fibrillation.

Strophanthus.—The action of strophanthus closely resembles that of digitalis. As an alternative method of producing a rapid effect upon the heart an intravenous injection of 1/200 to 1/100 gr. strophanthin may be given, provided that no digitalis has been administered within the last few days. The risk of producing toxic symptoms must always be borne in mind, as when the drug is introduced by the venous route the safety-valve action of the stomach is of necessity eliminated.

Quinidine.—This is a dextro-rotatory cinchona alkaloid, which was first used by Frey (35) in 1918 in cases of auricular fibrillation. Wenckebach (36) had previously, in 1914, obtained a temporary arrest of auricular fibrillation in one patient by giving 1 g. of quinine daily. Frey, in a series of twenty-two cases of fibrillation, obtained a restoration of normal rhythm in 50%. He gave the drug in doses of 0·4 g. (6 gr.) t.d.s. by mouth for three to eight days.

The administration of quinidine sulphate may be considered under the following headings :—

Selection of Case.—Quinidine is of greatest value in cases of auricular fibrillation, especially when there is no cardiac enlargement and no valvular disease (Cotton (37)). Best results are obtained in fibrillation of recent origin, and in those cases in which the symptoms increase with the onset of fibrillation. The infective group of cases is more suitable

than the degenerative. The drug can be tried in paroxysmal auricular fibrillation, as digitalis does not usually have any beneficial effect here (Parkinson and Nicholl (38)). It may be of value in auricular flutter, the normal rhythm being restored direct, without an intermediate stage of fibrillation. It is at times successful in those cases of flutter which have been converted to fibrillation by the use of digitalis, but which do not return to the normal sinus rhythm on discontinuing the drug. It does not appear to have any beneficial effect on premature systoles.

Contra-indications.—There are certain definite contra-indications which apply, no matter what the nature of the cardiac disorder may be. Thus quinidine must not be given in cases of heart failure. Such patients should first be treated with rest, digitalis and diuretics until compensation is restored. Then, after a week's cessation from the use of digitalis, quinidine may be given if thought advisable. Heart-block is also a contra-indication. It must not be given if there be a history of recent embolism. It is considered inadvisable to administer quinidine and digitalis simultaneously.

Regimen.—The patient must be kept in bed, and preferably at absolute rest, during the initial week of the treatment. This usually implies the necessity of skilled nurses. Attention must be paid to the bowels, and the diet should be such as will not give rise to flatulence.

Methods of Administration and Dosage.—The drug is given by the mouth in powder form in gelatine capsules. Probably the safest method is to give on the first day a test dose of 0·2 g. (3 gr.) to determine whether the patient has any idiosyncrasy to the drug. If no toxæmic symptoms appear, on the second day 0·4 g. (6 gr.) is given every three hours for four doses, and this is increased to five doses of 0·4 g. at three-hourly intervals on the third day. This dosage is then maintained until the end of the week, and if the normal rhythm has not been restored by this time the drug will probably

not prove successful. The pulse should be taken before each dose is given, and if it be found to be regular the drug should be discontinued, at any rate temporarily, until a tracing has been taken. Further indications for discontinuing the administration of quinidine are a slowing of the auricular rate to about 250 per minute, or an increase of the ventricular rate to over 160. In the former case there is a danger of the ventricle following directly the auricular rate. The exact rate of the auricle and ventricle are best determined by an electrocardiogram.

Hay (39) recommends that quinidine should be given every two hours by day in ten equal doses, as its action soon passes off. Starting with a test dose of 0·2 g. (3 gr.) on the first day, 2 g. (30 gr.) are given on the second day in ten doses of 0·2 g. each. If necessary, the dose may be increased to 3 g. (45 gr.) a day in ten doses of 0·3 g. (4·5 gr.). The smallest beneficial dose appears to be one of 5 gr., which restored the normal rhythm in a case of auricular fibrillation of recent origin, under our care, which was associated with a toxic goitre. As much as a total of 375 gr. has, however, been required before a satisfactory result was obtained, and it may be necessary to continue indefinitely with small doses of 5 gr. daily.

Symptoms of Toxæmia, and Dangers.—Frey (35), in 1918, drew attention to the possibility of quinidine idiosyncrasy, resulting in such symptoms as respiratory failure and cerebral paralysis. Benjamin and V. Kapff (40), in 1921, recorded instances of embolus occurring during the administration of quinidine. This is especially liable to follow restoration of normal rhythm, and is, in all probability, due to the detachment of a clot from the wall of the auricle. Embolus formation is not necessarily fatal. The onset of ventricular fibrillation may cause sudden death.

Headache is nearly always experienced while quinidine is being taken. Other toxic symptoms include sweating, nausea, vomiting, diarrhœa, abdominal pain and a scarla-

tiniform rash. Dimness of vision, or the occurrence of frequent ectopic ventricular beats, is an indication for the immediate discontinuance of the drug.

Results of Quinidine Administration.—In about 50% of cases of auricular fibrillation normal rhythm is restored by quinidine, but there is frequently recurrence even when the drug is given continuously.

The Action of the Drug.—In order to understand the action of quinidine upon the heart, it is necessary to explain the nature of the *circus movement* in auricular flutter and fibrillation. Thus in auricular flutter a wave circulates around the orifices of the venæ cavæ, and tangential or centrifugal waves are cast off from the mother wave to the outermost parts of the auricle. This constitutes the phenomenon known as the circus movement. In pure flutter this central wave travels very constantly over the same path. In auricular fibrillation a circus movement is also believed to occur, but the course pursued is uneven, and constantly altering from point to point, although the same broad path is followed. In both conditions a gap of excitable tissue exists between the head and tail of the circulating wave.

In a normally beating heart the impulse arises at the sino-auricular node, situated at the junction of the superior vena cava with the right auricle, at the upper part of the sulcus terminalis. Waves of excitation spread from this point over both auricles, passing radially in every direction from the node, and reaching the auriculo-ventricular node at the posterior and right edge of the auricular septum. Thence the impulses pass to the ventricle by the bundle of His. We are here concerned only with conduction in the auricle ; the waves of excitation radiate from the S–A node in concentric circles, like fluid spreading out when poured on a flat surface (Lewis). In auricular flutter and fibrillation it is believed that the excitation wave pursues a circular course, constituting the circus movement.

The nature of this circus movement and gap can be better

understood by referring to some experiments made on jelly-fish. A ring of tissue was cut from the umbrella of the fish, and by suitable electrical stimulation a contraction wave was started which circulated continuously round the ring for several days. A similar experiment was performed with rings of muscle cut from the auricles of fishes. The circular wave of contraction constitutes a circus movement, and must be studied a little more in detail. One would naturally expect a wave proceeding from the point of origin to spread circularly in both directions around the circle to meet at a point opposite to the point of origin. The two opposing waves

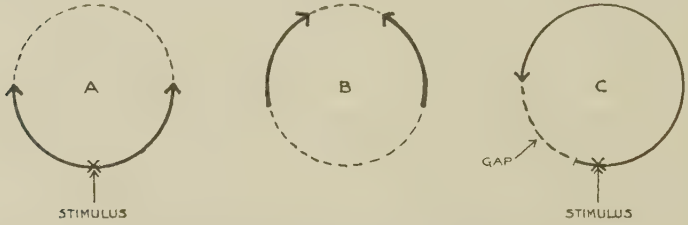


FIG. 34. The dotted line indicates excitable muscle tissue. The continuous line indicates muscle which is refractory, owing to contraction. The head of the wave of contraction is represented by the arrow-head.

would then mutually destroy one another and no continuous circus wave could be set up. With the aid of the diagrams shown in Fig. 34, the various facts required for comprehension of the nature of the circus movement and, consequently, of the action of quinidine can be appreciated.

If a single stimulus be applied at the point indicated by the cross in Fig. 34A, a wave of contraction will spread in both directions round the circle (see Fig. 34B), and on meeting will die away. The muscle which has just contracted remains in a refractory state for a certain time, and then excitability is restored. Restoration of excitability will begin at the point at which the stimulus was applied, and will spread round the ring in both directions in the wake of the contraction wave until the whole ring is once more excitable.

It is found experimentally that if stimuli be applied at a certain rate at the point indicated by the cross (see Fig. 34c), a wave of contraction can be produced which will circulate round the ring in one direction, a gap of excitable tissue always being present between the head and tail of the wave. It is important to note that with this rate of stimulation there is an initial spread of the excitation wave in the reverse direction to that of the main circulating wave, but this does not proceed far and dies out. In diagram C is shown the abortive wave which has travelled a little in a clockwise direction and has then stopped, and the main effective wave of contraction which has travelled in a counter-clockwise direction and has continued circulating.

It must now be explained why, when the rate of stimulation is gradually increased, a point is reached at which the waves do not pass from the site of stimulation in both directions, but behave as above described. When the stimuli are applied at a certain rate a state of partial refractoriness may occur, some of the muscle fibres in a particular strip of muscle being excitable, others refractory. If there be a large number of fibres in the refractory state in the portion of muscle on one side of the site of stimulation, and only a few fibres refractory on the other side, then contraction will take place in the less refractory muscle, but it will be blocked in the still more refractory zone on the other side. The contraction wave will therefore pass round the ring in one direction only, in the illustration given in a counter-clockwise direction, and the circus movement will be established. The direction of the circus movement in the example given depends therefore upon the relative refractoriness of the muscle on each side of the site of the stimulus. The gap of muscle between the head and tail of the wave is always excitable, and so the circulation is maintained. If, for any reason, this gap of muscle become inexcitable, the circus movement must stop, and this is known as " closing the gap."

The gap could be closed in three ways : 1. If the wave cir-

culate very fast, all the muscle will become refractory and there will be no excitable gap. 2. If the refractory period of the muscle be prolonged, when the head of the wave reaches the point of stimulation it will find it still refractory, and the wave will stop. 3. If the circulating wave take a shorter path, it will complete the circuit more quickly ; the first condition will thus be established and the movement must cease.

Factors which increase the gap on the other hand will tend to perpetuate the circus movement. They are the reverse of those just enumerated, *i.e.* : 1. A slower rate of conduction. 2. A shorter refractory period. 3. A longer path for the wave.

We are now in a position to understand the action of quinidine. It has been shown by Lewis and his co-workers (41), by experiments on dogs that quinidine will lengthen the refractory period of cardiac muscle up to twice its normal value. It will also slow the rate of conduction. Reference to the description given above on factors affecting the gap will show that prolongation of the refractory period tends to close the gap and abolish the circus movement ; whereas a slower rate of conduction and so of circulation of the wave tends to increase the gap and perpetuate the circus movement. The closure of the gap, the stoppage of the circus movement, and the abolition of flutter or fibrillation, must therefore depend upon which of these effects of quinidine preponderates in any individual case.

In addition to the above effects upon the cardiac rhythm, quinidine also slows the rate of auricular contraction but increases the rate of ventricular contraction. The auricular rate is slowed by the effect which the drug exercises upon the rate of conduction. The ventricular rate is increased for the following reasons : When the auricle is beating very rapidly a certain degree of block is produced, so that the ventricle does not respond to every stimulus. As the auricular rate is slowed this tendency to block is diminished and the ventricular rate is thereby increased.

In conclusion, the effect of quinidine upon heart muscle

can be summarised thus : The auricular rate is slowed. The rate of conduction in the auricle, ventricle and A-V junctional tissues is reduced. The absolute refractory period of auricular muscle is prolonged.

Quinidine has also some effect upon the vagus nerve, causing a partial paralysis which tends to increase the ventricular rate.

The electrocardiographic changes which may occur during quinidine administration are, according to Hay (39) : Slowing of auricular rate ; the onset of auricular flutter and 2 : 1 heart-block ; increase of ventricular rate ; appearance of ectopic ventricular beats ; restoration of normal rhythm in 50% cases of fibrillation.

The Value of the Drug.—This is very doubtful. as will be seen from what has been said above. The administration of quinidine has very definite dangers, and the cases in which it is worth a trial require careful selection, and very close watching during the treatment. Even if normal rhythm be established, the general condition of the patient is not always improved, while the underlying myocardial degeneration still remains.

The Continuous Administration of Oxygen.—Meakins (42) has shown that in auricular fibrillation, valvular disease of the heart, and in myocardial degeneration, there is no arterial anoxæmia if the lungs be normal. Oxygen will only give relief if pulmonary lesions be present, and will not benefit symptoms due to circulatory stagnation. Oxygen should therefore be used in those cases of heart failure accompanied by arterial anoxæmia.

There is, however, some evidence that oxygen " improves the contraction and aids the reintegration and recovery from fatigue of the heart muscle " (Starling (43)). The most efficacious and economical means of administering oxygen is by the continuous intranasal method of Bourne (44). A rubber catheter, smeared with 1% novocaine vaseline ointment, is passed into the nose to the level of the posterior

nares. The free end must not touch the posterior pharyngeal wall. The catheter is held in position by a head-piece consisting of a circular adjustable webbing band with an aluminium rod attachment for retaining it. The catheter is connected by a rubber tube with a Wolff's bottle containing water, through which oxygen is bubbled from a cylinder. The bottle is stood in a bowl of warm water. The rate of flow is adjusted so that about 70 to 80 bubbles pass each minute. The catheter is usually well tolerated, and the patient may sleep with it in position if necessary. By the use of this contrivance a sufficient supply of oxygen can usually be administered to relieve cyanosis.

Adrenalin in Adams-Stokes Attacks.—The hypodermic injection of atropine sulphate in doses of gr. 1/100 is the treatment which is usually recommended for the Adams-Stokes syndrome. Practically it is found that this drug is without effect, except perhaps in a few rare instances, in which vagal tone is very marked. Routier (45) has shown in animals that adrenalin has the power of increasing the rate of contraction of auricle and ventricle independently, and also of facilitating conduction along the auriculo-ventricular bundle. Phear and Parkinson (46), in 1922, recorded a case of complete heart-block in which Adams-Stokes attacks were occurring with great frequency. The subcutaneous injection of five minims of a 1/1,000 solution of adrenalin hydrochloride caused a rapid abolition of the seizures, although atropine had been previously tried without success. Feil (47), in 1923, had an equally successful result in the case of a man with complete heart-block, using ten minims of 1/1,000 adrenalin. The ventricular rate was not accelerated in these cases, but the occurrence of ventricular standstill was annulled.

Parkinson and Bain (48) in 1924, published the details of a further case which responded in a remarkable way to adrenalin injections. Here there appears to have been an acute lesion affecting the **A—V** bundle, causing variations in its

conducting power. At times there was complete dissociation between the auricle and ventricle which was slowly followed by recovery of conductivity. The Adams-Stokes attacks were most frequent during the latter phase. Adrenalin was injected in doses of five minims ; the attacks were abolished within three minutes and did not recur for periods of two to forty-eight hours. In this case, in addition to abolishing the periods of ventricular standstill, and thereby preventing the syncopal attacks, the rate of the dissociated ventricle was accelerated. It seems, therefore, probable that the adrenalin produces its effects by stimulation of sympathetic nerve terminations in the walls of the ventricle. Cullis and Tribe (49), in 1913, showed that the ventricles have many sympathetic nerve endings which probably reach them with the coronary arteries. Parkinson (48) concludes that adrenalin should be tried in every case of Adams-Stokes attacks, although from the examples he quotes he shows that it is not universally successful. Even in those cases which do respond it is probable that a time will come at which adrenalin loses its effect, and that then death is close at hand.

Adrenalin in Syncope.—The intracardiac injection of adrenalin in cases of sudden cessation of the heart-beat during the administration of an anæsthetic was recommended by Winter (50), in 1905, as the result of experiments upon animals. Zuntz (51), in 1919, recorded the first successful case of this treatment in man. Since then a review of the Continental and English literature shows that in a large number of instances syncope has been relieved and life saved by means of the intracardiac injection of adrenalin. It has been used not only for syncope occurring while under anæsthesia, but also for sudden cessation of heart-beat in diseases such as syphilitic stenosis of the coronary arteries (Bodon (52)). The adrenalin may be injected into the pericardium or into the wall or cavity of the left or right ventricle. Care has to be taken in making the injection to avoid damage

to certain important structures, especially the left lung, the internal mammary arteries and the interventricular septum. In order to avoid the risk of injuring the lung, with the consequent development of a pneumothorax, the injection is usually made into the right ventricle, as recommended by Petit-Dutaillis (53). The needle should be 8-10 cm. long and rather finer than that used in lumbar puncture. The variety of needle usually employed in regional anæsthesia is very suitable. The needle is attached to a syringe and 1 c.c. of 1/1,000 adrenalin hydrochloride sucked into it. It is then inserted into the fourth left intercostal space, close to the margin of the sternum and at the upper border of the fifth left costal cartilage. The artificial respiration which will have been performed while the injection is being prepared should be stopped in the position of expiration during the insertion of the needle, in order to retract the anterior margin of the lung as much as possible from the heart. The needle is pushed in at right angles to the chest wall for 2 or 3 cm., and then the point is turned slightly towards the mid-line. The resistance of the heart muscle is then felt, and on pushing the point a little further it enters the cavity of the right ventricle, when blood can be drawn back into the syringe. Petit-Dutaillis (53) prefers to inject the adrenalin into the cavity of the ventricle, and says that it is well absorbed by the endothelial lymphatics; other workers, such as Howell (54), recommend intramuscular injection. In any case the wound caused by the needle has no ill-effects, as judged by cases which have not responded permanently to the treatment and have been examined after death. After withdrawing the needle the præcordial region should be compressed a few times firmly and then artificial respiration recommenced.

The effect is usually almost instantaneous, the heart starting to beat within one or two minutes of the injection, and the radial pulse being perceptible within another two or three minutes. It may be an hour or longer before voluntary respirations are made in cases in which there has been some

little delay in giving the injection, and artificial respiration must be maintained until this is satisfactorily established. Howell (54) recommends the subcutaneous injection of 1/150 gr. of atropine, as in two cases the stagnation of the circulation led to the appearance of pulmonary œdema.

The Results.—Intracardiac injection of adrenalin appears to give better results than does cardiac massage through the diaphragm. Although the heart may be made to beat and breathing be restored, yet the patient may die after an interval of five or six hours without recovering consciousness. The sooner the injection is made, the more favourable is the prognosis.

REFERENCES

1. Potain.
Mém. d. l. soc. méd. d. Hôp. d. Paris, 1867, 3–27.
2. Mackenzie.
Journ. Path. and Bacteriol., 1892, i., 53–89.
3. Waller.
Journ. Physiol., 1887, viii., 229–234.
4. Einthoven.
Annal. der Physik. 1903, xii., 1059.
5. Cowan and Ritchie.
"*Diseases of the Heart*," 2nd edn., 1922, 72. Arnold & Co., London.
6. Gallavardin and Bérard.
Arch. des Malad. du Cœur, 1924, xvii., 18.
7. Goodall and Rogers.
Lancet, 1927, i., 486.
8. Russell.
"*The Sphygmometer*," 1921. Baillière, Tindall and Cox London.
9. MacWilliam and Kesson.
Heart, 1913, iv., 279.
10. v. Basch.
Med. Jahrb. Vienna, 1876, 431.
11. Hales.
Statical Essays, 1733, ii., 1. London.
12. Faivre.
Gaz. Méd. de Paris, 1856, 727.
13. Potain.
Arch. de Physiol., 1889.

14. Riva-Rocci.
Gaz. med. di Torino, 1896, li., 52.
15. v. Recklinghausen.
Arch. f. exp. Path. u. Phar., 1901, xlvi., 78.
16. Gallavardin.
"*La Tension Arterielle en Clinique*," 1922, 2ième édn. Masson et Cie., Paris.
17. Strasburger.
Zeit. f. klin. med., 1904, 371.
18. Ehret.
Münch. med. Woch., 1909, lvi., 606.
19. Korotkow.
Mitth. d. kais. mil. med. akad. zu Petersburg, 1905, xi., 365.
20. Ettinger.
Wien. klin. Woch., 1907, xx., 992.
21. Tixier.
Arch. des Malad. du Cœur, 1919, xii., 337.
22. MacWilliam and Melvin.
Heart, 1914, and *Journ. Physiol.*, 1914, xlviii., xxvii.
23. Warfield.
Arch. Int. Med., 1912, x., 258.
24. Pachon.
Compt. Rend. Soc. Biol., 1909, lxvi., 766.
25. Erlanger.
Amer. Journ. Physiol., 1902, vi., xxii.
26. Marey.
Trav. du Lab., 1876–77.
27. Rolleston.
Lancet, 1923, i., 519.
28. Withering.
"*An Account of the Foxglove*," 1785. Birmingham.
29. Eggleston.
Arch. Int. Med., 1915, xvi., 1, and *Amer. Journ. Med. Sci.*, 1920, clx., 625, and *Journ. Amer. Med. Assocn.*, 1920, lxxiv., 733.
30. Hatcher.
Amer. Journ. Pharm., 1910, lxxxii., 361.
31. Eggleston and Wyckoff.
Arch. Int. Med., 1922, xxx., 133.
32. Jensen.
Lancet, 1924, i., 747.
33. Canby Robinson.
Amer. Journ. Med. Sci., 1920, clix., 121.
34. Fraser.
Lancet, 1922, ii., 703.
35. Frey.
Berl. klin. Woch., 1918, lv., 417–450, 849.

36. Wenckebach.
Die Unregelmässige Hertzätigkeit, und ihre klinische Bedeutung, 1914, 125.
37. Cotton.
Proc. Roy. Soc. Med., 1922–23, xvi., 3.
38. Parkinson and Nicholl.
Lancet, 1922, ii., 1267.
39. Hay.
Quart. Journ. Med., 1922, xv., 313.
40. Benjamin and v. Kapff.
Deutsch. med. Woch., 1921, xlvii., 10.
41. Lewis, Drury, Iliescu and Wedd.
Heart, 1921–2, ix., 55.
42. Meakins.
Brit. Med. Journ., 1923, i., 1043.
43. Starling.
Lancet, 1921, ii., 1199.
44. Bourne.
Lancet, 1922, ii., 23.
45. Routier.
Compt. Rend. Soc. de Biol., 1915, lxxviii., 375.
46. Phear and Parkinson.
Lancet, 1922, i., 933.
47. Feil.
Journ. Amer. Med. Assocn., 1923, lxxx., 26.
48. Parkinson and Bain.
Lancet, 1924, ii., 311.
49. Cullis and Tribe.
Journ. Physiol., 1913, xlvi., 141.
50. Winter.
Wien. klin. Woch., 1905, xviii., 525.
51. Zuntz.
Münch., med. Woch., 1919, xxi., 562.
52. Bodon.
Lancet, 1923, i., 586.
53. Petit-Dutaillis.
Journ. de Chirurg., 1923, xxii., 519.
54. Howell.
Lancet, 1924, ii., 746.

CHAPTER IX

THE LUNGS

Artificial Pneumothorax

Historical.—Carson (1), of Liverpool, in 1822, first suggested that air might be admitted into the pleural cavity as a therapeutic measure in certain diseases of the lungs. Cayley (2), in 1885, at the Middlesex Hospital, London, was responsible for the establishment of a pneumothorax by pleural incision in a case of intractable hæmoptysis. Forlanini (3), in Italy, must undoubtedly be regarded as the originator of the modern pneumothorax treatment by puncture for pulmonary tuberculosis, his work dating back to 1882. Lillingston (4), in 1910, was one of the first to adopt this method in England.

The Indications

Collapse of the lung, by the introduction of air into the pleural cavity, is of value in certain cases of pulmonary tuberculosis, bronchiectasis, localised abscess of lung and interlobar empyema. It has also been used for the relief of pain in dry pleurisy, and for bronchopneumonia in children. In none of these diseases are the indications for such treatment sharply defined or universally accepted. Such being the case, a personal expression of view is permissible.

Pulmonary Tuberculosis. This subject has been reviewed by one of us (G. E. B. (5)), and it was found that in only about 2 to 5% of cases is artificial pneumothorax treatment indicated. Its induction should be considered under the following circumstances : In cases of unilateral disease which

remain pyrexial after absolute rest in bed for several weeks : In similar cases which, although responding to absolute rest, show renewal of signs of activity on moving, or on rising from bed : In cases of hæmoptysis which cannot be checked by other methods of treatment, and where the side from which the bleeding is occurring can be determined with a fair degree of accuracy : In unilateral acute pneumonic or broncho-pneumonic tuberculosis and in certain carefully selected cases of bilateral disease in which the activity is mainly confined to one lung.

Bronchiectasis.—The occurrence of a brilliant cure is especially likely in early cases before pleural adhesions have formed. This applies particularly to those cases resulting from the inhalation of some septic material, as may occur after operations upon the throat. Even in long-standing conditions of bronchiectasis, if unilateral, a determined attempt should be made to establish pulmonary collapse.

Interlobar Empyema and Localised Pulmonary Abscess.—Artificial pneumothorax may be considered in those cases in which an interlobar empyema or abscess has ruptured into a bronchus, and the pus is not completely expectorated. The sputum remains offensive, and constitutional disturbance is present as indicated by pyrexia. Here, again, pleural adhesions may render the operation impossible or unsuccessful, as only a partial collapse is obtained. In such an instance an attempt may be made to cauterise the adhesions with the use of a thoracoscope.

The Method

The requirements are as follows :

Pneumothorax Apparatus.—There are several types in use. The Lillingston and Pearson apparatus is that which we usually employ. It has the advantage of simplicity, and can be used both for inserting air and also for removing it in cases of spontaneous pneumothorax.

The arrangement of the pneumothorax apparatus can be seen by a reference to the diagram, and a detailed description is not necessary (see Fig. 35). A and B are glass bottles, B being graduated in c.c. from 0 to 1,100. C is a glass manometer. D is a fourway glass connection. E is a glass connection. F and G are glass filters containing sterile wool.

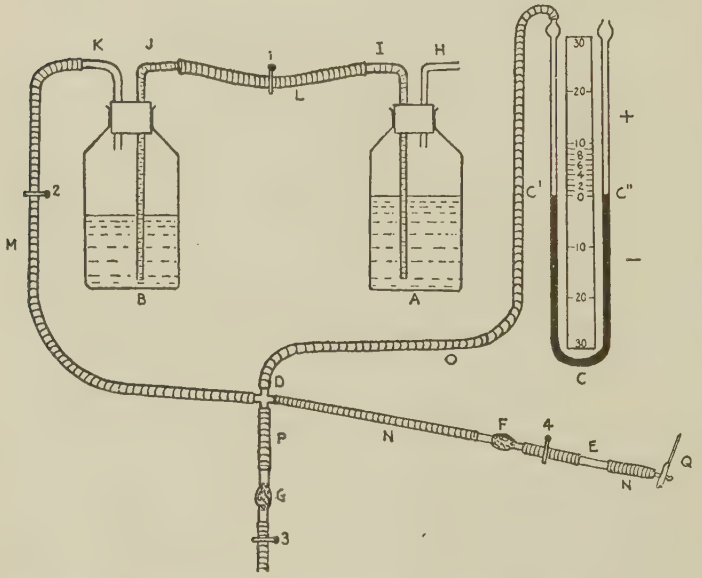


Fig. 35.—Diagrammatic Representation of Modified Lillingston and Pearson Pneumothorax Apparatus. The bottle B is graduated in c.c. from 0 to 1,100.

H, I, J and K are glass tubes. L, M, N, O and P are rubber tubes. Q is the needle. 1, 2, 3 and 4 are clips on the rubber tubes.

Pneumothorax Needles.—The needle used for the primary induction is often referred to as the No. 1 needle. That form, devised by Clive Riviere, is commonly employed. It consists of a blunt-ended cannula with a thin edge for penetrating the pleura, and a trocar for piercing the tissues of the chest

wall. The needle used for refills is of a different pattern ; it may be referred to as the No. 2 needle. The Saugman pattern is the one we use. This is shorter than the No. 1 needle and has a bevelled cutting edge like an ordinary hypodermic needle. It is provided with a stilette for clearing purposes. The needles are illustrated in the text (see Figs. 36 and 37).

A 2 c.c. syringe and hypodermic needle of sufficient length

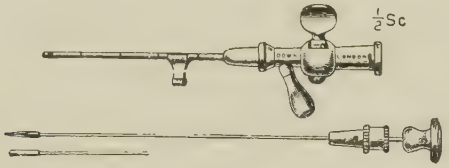


Fig. 36.—Clive Riviere Needle (No. 1 Needle).

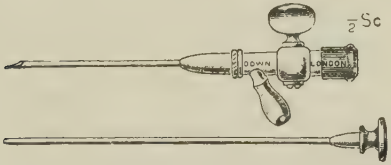


Fig. 37.—Saugman Needle (No. 2 Needle), with Rubber Cap to act as a Stuffing-box.

to reach through the chest wall to the pleura. (This distance is rarely more than 3 cm.)

Sterile novocaine solution ($\frac{1}{3}$ gr. in 10 m.).

Iodine, spirit lamp and sterile towels.

Some restorative, such as brandy, strychnine or pituitrin, should be at hand.

Preparation of the Apparatus.—The glass bottles, rubber tubes, glass tubes and glass filters are all sterilised by boiling. The needles, placed in a tin, are sterilised by dry heat. The manometer is filled to the zero point with water coloured with a little red ink. This does not stain the glass and makes readings easy. The glass filters are filled with sterile cotton

wool. The tubes are placed in position. Bottle A is filled with coloured solution of perchloride of mercury (1/1000), or with 1/100 carbolic acid. The fluid is passed over to bottle B, by blowing a little air into bottle A with a Higginson syringe attached to the tube H, which starts the siphon action. With the clips 1, 2 and 3 open the fluid in the bottles can be now run from one to the other by raising or lowering one of them. Half the fluid should be left in bottle B and half in A. The apparatus should be examined to see that there are no leaks before use. This can be done by closing clips 3 and 4 and opening clips 1 and 2. If, now, bottle A be raised, air will be driven from bottle B into the manometer and the fluid in limb C′ depressed. The level in the manometer should remain constant as long as the bottle A is kept in the same position. In the same way, if bottle A be lowered the fluid in limb C′ will rise. It can easily be ascertained that air can be driven through the pneumothorax needle by opening clips 1, 2 and 4 and closing 3. If the end of the needle be now put under methylated spirit, having withdrawn the trocar and closed the side tap, air will be seen bubbling from the end of the needle on raising bottle A.

Preparation of the Patient.—The patient is in bed. He should be given an aperient the night before the operation, and the induction should not be made immediately after a meal. Half an hour before the operation an injection of $\frac{1}{3}$ gr. omnopon should be given, in order to calm the patient and diminish any tendency to pleural shock. If the pneumothorax is to be induced on the right side of the chest the patient lies on his back turned towards his left side, with the right arm forward above the head, which is kept low, and a pillow is placed under the chest and left shoulder, so as to widen the intercostal spaces. The site of election for the first puncture is in the sixth space in the anterior axillary region. The skin over this area is painted with iodine, and sterile towels placed on the bed-clothes and against the chest.

The Operation.—The novocaine solution is injected, about

2 c.c. being used. The skin over the intercostal space is held taut with two fingers of the left hand. The needle is then inserted obliquely, just in front of the fingers, and a little novocaine injected intradermally to cause a bleb. After a short pause the needle is moved into a position at right angles to the chest wall, and gradually pushed through the intercostal space. Novocaine is slowly injected as the needle moves downwards to the pleura. Care should be taken to inject the solution right down to the pleura, as this probably plays a part in the prevention of pleural shock which may cause a fatal result.

The patient must now be warned against coughing during the operation. If the desire to cough be irresistible, due warning must be given, otherwise the sudden increase in the pleural pressure when the patient coughs, with the pneumothorax needle in the pleural cavity, would drive the fluid out of the manometer. This can be prevented if warning be given by squeezing the tube N, leading from the needle to the manometer, or by closing the clip 4.

The No. 1 needle is now taken out of the sterile tin and passed through the flame of the spirit lamp, the trocar being drawn in and out to make sure that it is working properly. The rubber tube N leading to the manometer is attached to the side arm of the needle. Clips 1, 2 and 3 are closed and clip 4 opened. With the trocar in position the needle is pushed through the skin at the anæsthetised spot, and through the intercostal muscles, for about 1 cm. or less if the patient be very thin. It must be held firmly in the hand, with the top of the trocar in the palm, and not in the position of a pen between the fingers. The trocar is now withdrawn and the stopcock turned, so that the lumen of the needle is in communication with the tube leading to the manometer. The cannula is now pushed on through the remainder of the intercostal muscle down to the pleura.

When it is in contact with the pleura, but not through it, an oscillation will probably be observed in the manometer,

not directly synchronous with respiration and of small extent. The cannula is now pushed through the pleura, which can often be felt to give way with a snap. Great care must here be taken not to penetrate the lung. When the cannula is in the pleural cavity the fluid in the limb C″ of the manometer falls below zero and oscillates with respiration. These oscillations correspond accurately with the respiratory movements, falling with inspiration in the limb C″ of the manometer and rising with expiration. A reading is now taken, each centimetre of fall of fluid in the limb C″ corresponding with a change of pressure in the pleural cavity of 2 cm. of water. The oscillations should be at least 3 to 6 cm., and are often as much as 10 cm. Thus a reading of — 10, — 2 may be obtained, giving a mean negative intrathoracic pressure of — 6 cm. water. The respiratory fluctuations are greater when the patient takes a very deep breath, but the reading should be taken with ordinary moderately deep breathing. The needle may become blocked during its passage through the deeper parts of the chest wall, in which case it can be cleared with the stilette which is supplied with the No. 1 needle. The visceral layer of the pleura may also come in contact with the end of the cannula and cause obstruction. Letting in a small quantity of air by opening clips 1 and 2 is the best means of dealing with such a condition. When it is certain that the end of the cannula lies free in the pleural cavity, air may be admitted. This is accomplished by opening the clips 1 and 2, when the air is sucked from the bottle B into the chest, and fluid passes over from bottle A into bottle B to take its place. When about 100 c.c. have thus passed in by suction, the bottle A can be raised slightly and another 200 c.c. of air admitted. The clips 1 and 2 are then closed, and the manometric readings taken for inspiration and expiration, with the patient breathing quietly and moderately deeply as before. They will still be negative, unless the air has been injected into a small loculated pleural space. These readings may be — 8, — 1. The

mean pressure would in this case be — 4·5. The pressure changes, and the amount of gas injected, would then be recorded as follows : — 6, 300 c.c., — 4·5. It is to be noted that with this pneumothorax apparatus readings of intrapleural pressure cannot be taken while air is flowing in. The clips 1, 2 and 3 must be closed in order to obtain a reading. The needle is now withdrawn from the chest and a little iodine again applied to the site of the puncture. A collodion dressing is not usually required. If there be a tendency to cough, a large pad and a firm chest binder should be used in order to minimise extravasation of air. The patient is kept in bed at absolute rest for the first month of pneumothorax treatment.

Refills.—The same apparatus is required as for the initial pneumothorax operation, but a No. 2 needle (Saugman) is used. The patient is prepared as before, but it is not usually necessary to give a preliminary injection of omnopon unless he be very nervous. It is wise, however, not to omit the anæsthetisation of the pleura with novocaine, as death may follow from pleural shock in refills as well as during the primary induction. A spot close to that used for the induction is chosen. The No. 2 needle is taken from its sterile gauze, flamed, and the stilette slightly withdrawn, so that it does not protrude beyond the point. The rubber tube leading to the manometer is attached to its side arm. The pneumothorax apparatus has already been prepared with the fluid in bottle B up to the 0 mark, the remainder being in bottle A. The clips 1, 2 and 3 are closed. The needle is now pushed straight through the chest wall at the anæsthetised spot into the pleural cavity. Directly it is felt to enter, the stilette is withdrawn and the stopcock closed, so that the lumen of the needle is in communication with the manometer. The fluid in the manometer will now oscillate. The pointed needle is used, as the air introduced at the primary induction (on the previous day) will prevent the lung being injured.

The reading is then taken. This will be found to be lower than the final reading at the previous injection, as some of the air introduced has been absorbed. Clips 1 and 2 are now opened and air allowed to enter the pleural cavity. After about 200 c.c. have passed in, the clips should be closed and a reading taken to make sure that the needle is in the pleural cavity and that the pressure is not rising too quickly. About 400 or 500 c.c. will probably be enough for the first refill, in order to reduce the negative pressure in the pleural cavity by about 2 or 3 cm. of water. Thus the readings at the first refill may be — 5·5, 400 c.c., — 2. If there be many adhesions, and the lung be not collapsing well, a smaller quantity of air should be required to reduce the negative pressure to a similar degree. The needle is then withdrawn.

The spacing of refills, and the determination of the correct amount of air to be injected, is a matter of extreme importance, and is comparable with the administration of a vaccine. In neither case is it a matter of routine, in each the personal factor of the patient is of paramount importance. Usually the second refill is given two days after the first, and the third three days after the second. The interval can then be gradually lengthened to a week, ten days, a fortnight, three weeks and a month. High intrapleural pressures should be avoided, as they cause mediastinal displacement, possibly tend to promote pleural effusion, and seem to have a direct relation to loss of weight on the part of the patient (Burrell and Garden (6)).

If there be a rise of temperature after the initial induction, the first refill should not be given the next day. The temperature should be allowed to subside first. A rise of temperature just before a refill is due, probably indicates that the refills are being given at too long intervals, and that the lung is starting to re-expand (Burrell (7)). A rise of temperature after a refill is sometimes an indication that too much air has been injected. An X-ray should be taken at the end of the second week to determine the degree of pulmonary

collapse, and the subsequent treatment should be subjected to X-ray control, in order to determine what degree of collapse is necessary to produce the optimum effect, as judged by temperature, pulse and body weight, and also so that the range of intrapleural pressure which produces such collapse can be ascertained. It is not usually necessary to use a positive pressure to produce satisfactory collapse.

Difficulties and Some Complications.—The manometer has been described as the heart of the pneumothorax apparatus. It is certainly of vital importance, not for the actual introduction of the air, but for its safe introduction.

The character of its oscillations affords the only reliable guide as to whether the end of the needle lies free in the intrapleural space. These typical movements have already been described.

Certain abnormal events may occur, in which case no air must be admitted into the chest :—

A negative pressure may be obtained on inserting the needle into the chest, but the fluid in the manometer may show this as a stationary negative pressure, without any respiratory excursions. This indicates that the needle has been in the pleural cavity, but is now either blocked or in the lung. The negative pressure may increase for a little with each inspiration, without any oscillations occurring if the needle be in the lung. The pressure may be slightly positive during expiration at the primary induction and negative during inspiration, indicating that the needle is in a bronchus or small cavity in the lung. The manometer may show a positive pressure which rises slowly or rapidly, indicating that the needle is in a blood vessel. The blood may then be seen passing through the glass connection E in the rubber tube attached to the needle. The pressure may be negative, but show irregular respiratory oscillations if the needle be in a pleural cavity, much loculated by adhesions.

From such examples as these, it will be realised how impor-

tant it is to be certain that the manometer indicates that the needle is in the pleural cavity before any air is admitted.

Complications.—*Pleural shock* may occur, especially if the patient be very nervous and if the pleura be not anæsthetised.

Surgical emphysema may develop within a few hours of the injection of air, either as the result of coughing or because of injury to the lung, or because the air has been injected extrapleurally between the pleura and the intrathoracic fascia. If a lung, which is bound down to the chest wall by adherent pleura, be punctured, surgical emphysema may develop without the introduction of any air.

Gas Embolus.—This may occur from introduction of air into a vessel, especially a pulmonary vein, in which the pressure is negative.

Effusion.—About 50% of pneumothorax cases develop an effusion in the pleura during some time of their treatment. This may be : *Small*, giving rise to little constitutional disturbance, and often unrecognised except by X-rays. *Large*, causing constitutional disturbance with pyrexia. The fluid is serous, rich in cells and frequently contains tubercle bacilli. It indicates an active tuberculous pleurisy. *Infective*, often accompanied by rigors. This is pyogenic in origin.

In any case pleural adhesions are likely to occur, and it is improbable that a satisfactory collapse will be obtained afterwards, unless the fluid be removed and replaced by air.

The Treatment of Spontaneous Pneumothorax

In cases of spontaneous pneumothorax in which pressure symptoms are urgent, it has been the custom to allow the air to escape through a needle inserted into the pleural cavity.

The use of the Lillingston and Pearson apparatus enables the intrathoracic pressure to be determined before and after the air is removed, and also gives a reading of the volume of air that has been abstracted. It may therefore be employed with advantage.

For this purpose the bottle B is filled with fluid and the bottle A is almost empty. The chest wall and pleura are anæsthetised with novocaine, as for pneumothorax induction, and a No. 1 needle inserted into the pleural cavity. The intrathoracic pressure is then read, the clips 1, 2 and 3 being shut and 4 open. A reading such as — 8 + 10 may be obtained, indicating a mean pressure of + 1. The fluid in bottle B is now allowed to run over by siphonage into bottle A by opening the clips 1 and 2, and air from the chest replaces the fluid in the bottle B, the air in bottle A being expelled through tube H. Sufficient air should be removed to relieve the intrathoracic tension and produce a final pressure, which is slightly negative. In the example quoted, after removal of 400 c.c. of air, the readings were — 8 + 6, a mean of — 1. The needle is then removed from the chest.

If the pressure subsequently rise, as is often the case with a valve-opening in the lungs, more air can be removed in a similar manner on a subsequent occasion. In cases of spontaneous hydropneumothorax, the fluid can be simultaneously removed through an aspirating cannula inserted into one of the lower intercostal spaces.

It must be remembered that, after removal of gases from a pleural cavity, the whole of the pneumothorax apparatus will require sterilisation before it is used for another patient. This is because the gases from the pleural cavity have passed into the bottle B. The bottles, tubing, and filters should be dismantled and boiled. The filters are then refilled with sterile wool and fresh perchloride solution put into the bottles.

Gas Replacement of Pleural Effusions

On April 25th, 1882, Parker (8) demonstrated to the Fellows of the Royal Medical and Chirurgical Society, London, an apparatus for injecting filtered and carbolised air into the pleural cavity in order to facilitate aspiration in cases of empyema. He also referred to a case which had been success-

fully treated by this means. Judging from the subsequent discussion, the suggestion was not very favourably received. The operation is now, however, a recognised and valuable method of treatment for certain cases of pleural effusion.

Gas replacement consists in the removal of fluid from the pleural cavity through one of the lower intercostal spaces towards the back, and, at the same time, the introduction of air or nitrogen to take its place through one of the intercostal spaces higher up and in front. Nitrogen gas does not appear to be absorbed quite so quickly as air.

This treatment is of most value in cases of tuberculous pleural effusion, but is at times used for pneumococcal empyema, and for effusions due to malignant disease. In tuberculous pleural effusions it is of value for the following reasons :—

The lung is not allowed to re-expand. Any pulmonary tuberculous focus is thereby kept at rest and adhesions are prevented from forming between the two pleural layers. These would probably render the subsequent establishment of an artificial pneumothorax impracticable.

When the mediastinum has been severely dislocated by a large effusion, the removal of the whole of the fluid may not be possible owing to symptoms of shock appearing as the heart passes back to its normal site. Gas replacement allows the fluid to be removed without a sudden readjustment of the mediastinum.

The pleural cavity can be almost completely emptied. This tends to lessen the probability of basal adhesions subsequently forming.

Gas replacement diminishes the tendency to the re-formation of pleural effusion, which so frequently takes place after simple aspiration. The method is therefore of value in the treatment of chronic recurrent pleural effusions. This is strange when it is remembered how often a pleural effusion complicates the establishment of an artificial pneumothorax.

Pneumococcal empyema may, in some instances, be successfully treated by this method without recourse to an open operation, as has been advocated by Chandler (9). It must

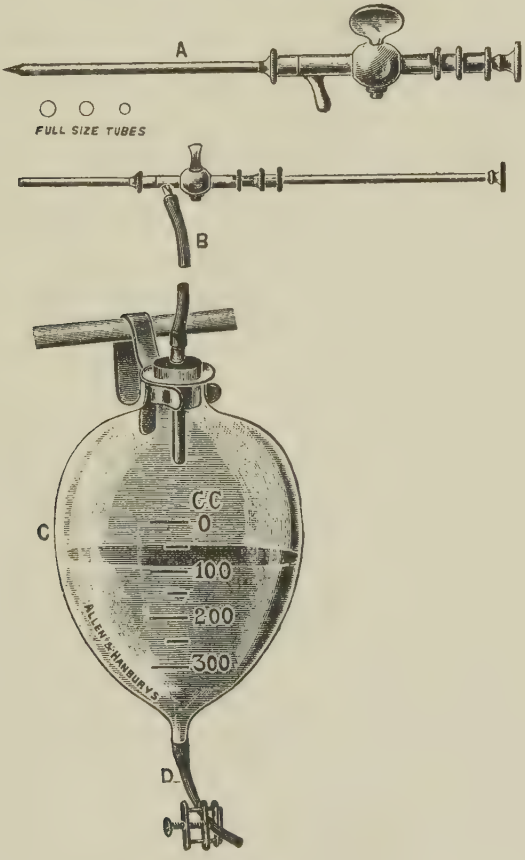


FIG. 38.—Burrell's Aspirator.

be impossible to remove by aspiration the large clots of exudate which are almost invariably present.

In suspected cases of malignant disease of the lung, mediastinum or pleura, complicated by a pleural effusion, a

gas replacement of the fluid is of value in allowing a clearer X-ray picture to be taken for diagnostic purposes. It probably does not relieve pain, if withdrawal of the fluid alone failed to do so, although it is sometimes used for this purpose.

The fluid may be removed by a Dieulafoy 100 c.c. glass aspirating syringe with two taps, or by a Potain's aspirator. It may also be allowed to drain away by simple siphonage through a needle and rubber tube. The *improved aspirator*, described by Burrell (10), possesses several advantages over the older methods, and is simple to use.

Apparatus.—Burrell's aspirator with Potain's trocar and cannula in three sizes (see Fig. 38).

Pneumothorax apparatus with No. 1 needle (Riviere).

Hypodermic syringe and needle.

Iodine, gauze and collodion.

Sterile novocaine solution ($\frac{1}{3}$ gr. in 10 m.).

The aspirator bottle and tubes are first sterilised by boiling. The lower tube D is clamped off, and boiled water is poured into the bottle until it is about one-third full. The cork and tube B are put into its upper end, and it is then hung on the side of the bed. It is important to see that all the connections are air-tight, or else aspiration will not take place.

Operation.—The skin over one of the intercostal spaces is painted with iodine at the site judged to be most favourable for the removal of fluid, either in the posterior axillary line or in the mid-axilla. The patient, lying on the sound side, is propped up slightly with pillows. The skin, muscles and pleura are now anæsthetised with 2 c.c. of the novocaine solution, as for pneumothorax induction. The pulse rate is counted. The Potain trocar and cannula is now introduced, the rubber tube from the upper opening of the bottle being first attached to the side arm of the cannula. When the trocar and cannula have pierced the pleura, the trocar is withdrawn to its full extent, and the stopcock turned so that the lumen of the cannula is in direct communication with the bottle. The clamp from the exit tube D of the bottle is

opened, and, as the water runs out into a receptacle on the floor, the fluid passes from the chest into the bottle to take its place. A continuous stream is thus established from the chest to the bottle, and from the bottle to the receptacle, but the level of fluid in the bottle remains constant. When a satisfactory flow is thus obtained, it is stopped temporarily by clamping the tube, while a No. 1 pneumothorax needle, attached to the tube of the artificial pneumothorax apparatus, is inserted into the pleural cavity in front, above the level of the effusion. The intrapleural pressure is then recorded. If this be high, above a mean of + 4 cm. water, fluid is run out of the chest to lower it before any air is introduced. Air is now passed in to take the place of the fluid running out. The rate of the admission of the air is so regulated, by raising or lowering the bottle A, that the intrapleural pressure is kept about constant.

If by any chance the pneumothorax needle should have been inserted into fluid, either free or pocketed in the pleura, the fluid will be observed passing through the glass connection (E) in the rubber tube (N) attached to the pneumothorax needle. It can be prevented from reaching the filter F by closing the clip 4. Before allowing air to enter now it is advisable to run off some fluid through the aspirating cannula. The pulse should be taken from time to time to see that it does not increase in rate unduly. If this occur the operation should be stopped. When all the fluid has been run off, the intrapleural pressure should be left slightly lower than it was at the beginning. It will usually be found that less air has been introduced than fluid withdrawn, even if the final pressure be equal to the original one. On removal of the aspirating cannula, a gauze and collodion dressing should be applied.

Lipiodol in Diseases of the Lungs

It is not possible to visualise clearly the outline either of the bronchi and their ramifications or of pulmonary cavities

by means of a direct X-ray examination. If, however, some substance opaque to the rays be introduced into the bronchi, their configuration is clearly revealed. Chevalier Jackson (11) in 1918 insufflated for this purpose a bismuth powder through a bronchoscope, and Clerf (12) later reported two cases in which he used this method and outlined the bronchi with a dry powder of bismuth subcarbonate. He advises that only one lobe of a lung should be delineated at a time, and insufflates about two drachms of powder. A bismuth paste, injected into a bronchus through a bronchoscope, was also used for this purpose by Lynah and Stewart (13) in 1920. The chest was then quickly X-rayed, as the bismuth is easily disturbed by coughing. In this way the outline of a bronchiectatic cavity was clearly shown. The preparation which Lynah used was a paste made of one part of bismuth subcarbonate in two parts of olive oil ; it was sterilised by heat before injection, and about 8 c.c. were introduced.

The use of bismuth has now been superseded by a French preparation known as lipiodol. Lipiodol is a compound of iodine in poppyseed oil, which contains 40% iodine. It is a transparent oil of a light brown colour, which is opaque to the X-rays, and so heavy that it sinks in water. On prolonged exposure to air it darkens, and should not then be used for injection. Sicard and Forestier (14) in 1922 injected it into the trachea in man, using either a cannula introduced into the glottis with the aid of a laryngoscope, or else passing it through a curved needle inserted through the crico-thyroid membrane.

It had previously been shown that lipiodol, despite its high iodine content, is non-toxic, and produces no reaction when injected subcutaneously, intramuscularly, or even intravenously. After injection into the bronchi, the greater part of the lipiodol is removed from the lungs by expectoration, but it is also dissociated by the pulmonary tissues and the iodine is carried in the general circulation and excreted partly in the urine and partly in the saliva. Lipiodol remains for

some time in a lung which has previously been collapsed by an artificial pneumothorax, as the expulsive action of coughing is thereby abolished.

Sergent and Cottenot (15) in 1923 performed important work on the subject, using lipiodol for outlining cavities in the lung. Their method was to introduce a curved needle, the calibre of that used for lumbar puncture, through the crico-thyroid membrane, and first anæsthetise the trachea by the injection of 3 or 4 c.c. of 2% cocaine. After a few minutes the needle is connected by a stout rubber tube to a syringe containing the lipiodol, of which 20 to 40 c.c. are slowly injected into the trachea.

Armand-Delille, Duhamel and Marty (16) in 1924 published their experiences of the method in the diagnosis of certain pulmonary affections of children. They employed a curved trocar and cannula, which was inserted through the crico-thyroid membrane, and held in position by tapes tied round the neck, attached to a flange on the cannula fitting the neck like a tracheotomy tube. The advantage of this instrument is that it is not likely to become displaced if the child move, swallow or cough, and so the subsequent injection of the lipiodol is facilitated. About 8 to 10 c.c. of lipiodol are sufficient to outline the bronchi in a child of seven to fourteen years of age. Similar observations on children have been published in America by Armand-Delille and Gelston (17), and in England by Armand-Delille and Moncrieff (18).

A straight needle attached to a syringe may also be used for the injection, and this method is now frequently employed, the lipiodol being introduced either through the crico-thyroid membrane, or directly into the trachea below the cricoid cartilage as is done by Tudor Edwards. The latter site for the injection appears preferable as there is less risk of the lipiodol irritating the larynx than if it be introduced through the crico-thyroid membrane.

The Indications.—X-ray photographs of the lungs after

intratracheal injection of lipiodol afford a graphic representation of the position of the trachea and main bronchi, of cavities in the lung connected with patent bronchi, of bronchial and pleural fistulæ, and of the relative permeability of the bronchial fields in different portions of the lungs.

Thus, it will show displacement of the trachea and bronchi due to pulmonary fibrosis or other causes, the presence of bronchiectasis in its various stages, and whether or not the lumen of the bronchi is obliterated by obstruction within or without, such as that caused by a foreign body, new growth or fibrosis. Lipiodol injections are chiefly of value in suspected cases of bronchiectasis, to determine whether there is a cavity present, and, if so, the extent of lung involved. They also show the condition of the apparently sound lung. This is of great importance in the treatment of bronchiectasis, especially in those cases in which it is impossible to establish an artificial pneumothorax on account of pleural adhesions, and in which a surgical operation, such as thoracoplasty, is contemplated. A thoracoplastic operation for bronchiectasis would generally be contra-indicated if the lipiodol injection showed that the opposite lung was also the seat of bronchiectatic changes. In children, lipiodol injections are chiefly of value in distinguishing between cavities due to bronchiectasis and those caused by tuberculosis. In definite cases of tuberculosis it is considered by some inadvisable to inject lipiodol, for fear of increasing the activity of the disease.

The X-ray appearances of the lungs before and after lipiodol injection are shown in the accompanying plates (see Figs. 39, 40, 41 and 42). The alteration in the appearances of the shadows in bronchiectasis in the erect and supine positions owing to the presence of gas and fluid is well shown. This affords definite radiological evidence that cavitation is present (see Figs. 41 and 42). Fig. 40 shows the bronchial tree outlined with a thin layer of lipiodol; it was taken with the patient lying down, and the amount injected was not nearly sufficient to fill the bronchi.

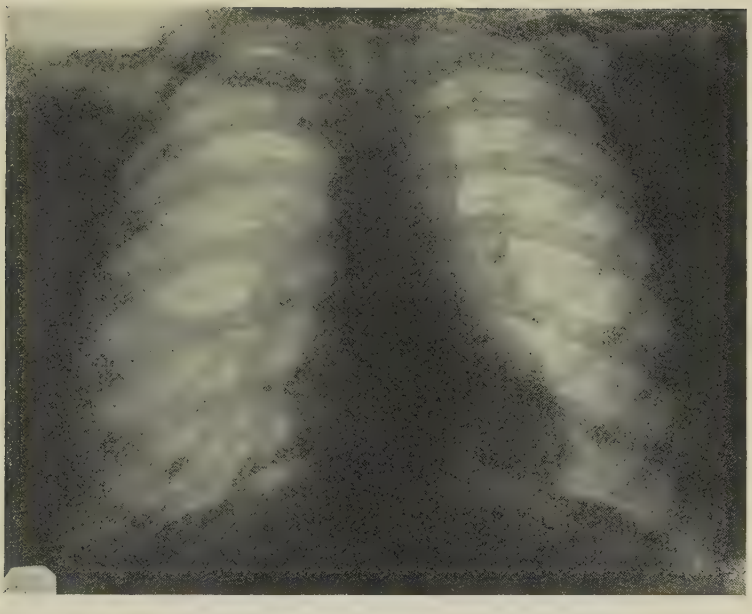


FIG. 39.—Radiogram of chest before injection of lipiodol.

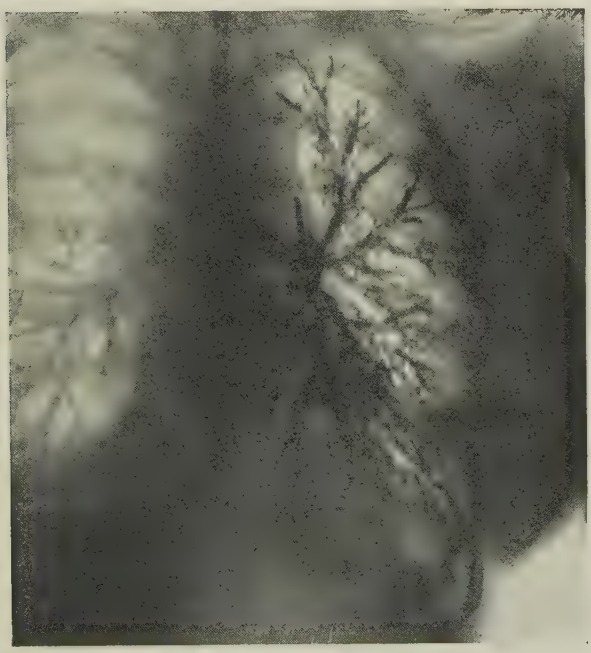


FIG. 40.—Radiogram of chest shown in Fig. 39 after injection of lipiodol.

[*To face p.* 306.

Fig. 41.—Radiogram of chest after lipiodol injection, showing cavities containing fluid and gas (erect position).

Fig. 42.—Radiogram of same chest as shown in Fig. 41, cavities now filled with lipiodol (recumbent position).

Apparatus.—Minim syringe with a fine needle (No. 7, hypodermic).

Minim syringe with a stouter needle (No. 2, hypodermic).

Lipiodol syringe. This must be strong, as considerable pressure is required to force the oil through the needle, and it should be furnished with a finger support to aid injection. A special pattern is made by Allen and Hanbury, in which a straight needle (No. 17 B.W.G., 1½ inches long), screws on to the syringe, and the piston moves either by a slide or screw action, controlled by a small and adjustable cap. The slide motion of the piston is for filling the syringe through a special wide-bore cannula, and the screw action facilitates injection, as there is less risk of displacing the point of the needle from the lumen of the trachea than if the piston be pushed down directly by hand. By reversing the screw action the piston can be withdrawn a trifle to suck back a bubble of air. This is often required to make sure that the needle is still in the trachea.

Sterile novocaine solution, 1%.

Sterile cocaine solution, 5%.

Lipiodol warmed in hot water bath.

Iodine and wool.

The Operation.—Preliminary tests should be made to determine whether the patient is sensitive to iodides or cocaine. Ten grains of potassium iodide are given by mouth three times a day, for one day, to see if iodism is produced, as judged by lachrymation and nasal catarrh. Lipiodol does not produce toxic effects in patients intolerant to iodides unless it enters the stomach. If the patient be found sensitive, it is wise to inject only 15 c.c. into the trachea. The stomach should also be screened when the lungs are X-rayed, and if any lipiodol has been swallowed it can be removed quickly by a stomach wash-out. The next day ⅛ gr. cocaine hydrochloride is injected subcutaneously, idiosyncrasy to the drug being shown by faintness, dyspnœa, palpitations or cramps. If these tests be satisfactory, the

operation can be performed the day after. If the patient be of a nervous temperament he should receive a preliminary injection of $\frac{1}{3}$ gr. omnopon half an hour previously. He is placed in a semi-sitting position, the head straight, neck extended and propped up by pillows. Subsequent movements of the patient are facilitated if he be on an adjustable operating table. The skin of the neck is painted with iodine, the position of the cricoid cartilage identified and the skin and subcutaneous tissues down to the trachea anæsthetised by injecting $\frac{1}{2}$ c.c. of novocaine through the fine needle; 10 to 12 m. of cocaine, warmed to 100° F., are then injected into the trachea through the stouter needle. For this purpose the cricoid cartilage is steadied with the left hand, and the needle attached to the syringe containing the cocaine is pushed at right angles to the skin into the trachea just below the cricoid cartilage. There is usually no difficulty in avoiding the cartilaginous rings, the needle can be felt to enter the trachea, and on withdrawing the piston slightly, the passage of a few bubbles of air into the syringe will show that the point of the needle is free in the lumen of the trachea. The cocaine is then rapidly injected and the needle withdrawn at once so that it is not broken when the patient coughs. The cough causes the cocaine to be spread over the surface of the trachea and lower part of the larynx. The lipiodol, which has in the meanwhile been warmed to body temperature in a water bath, is drawn up into the special syringe, the needle attached, and quickly introduced into the trachea just below the cricoid cartilage as for the injection of the cocaine. The piston is withdrawn a little and a bubble of air sucked back to verify that the needle is in the correct position. Owing to the size and direction of the right main bronchus, there is a tendency for the lipiodol to pass into the right lung if the patient lie on his back. If it be desired, however, to inject the left bronchial field, the patient must be turned to the left side as soon as the needle is in the trachea. He should, however, be kept in the semi-sitting

posture, and the position of the point of the needle verified by again drawing back a little air before the injection is begun.

Twenty to thirty c.c. of lipiodol are then slowly injected by depressing the piston, keeping the syringe quite steady during this operation and from time to time withdrawing a bubble of air if there be any doubt whether the needle is still in the trachea. In order to inject the apical bronchi the lipiodol must be allowed to run below the bifurcation of the trachea before the patient is tilted to one side and the head lowered. This usually takes from two to three minutes. After remaining in the required position for five or ten minutes, he is taken to the X-ray room and photographed first in the supine and then in the erect position.

Difficulties and Some Complications.—There is not usually difficulty in entering the trachea, but small children may require a general anæsthetic to prevent cough. If the trachea has been properly anæsthetised by cocaine, coughing does not usually occur. The chief difficulty is to keep the point of the needle free in the lumen of the trachea while the requisite pressure is being exerted on the piston to inject the oil. If this be not done, the lipiodol may be injected into the tissues around the trachea and may thereby produce compression with tracheal dyspnœa.

Such complications as idiosyncrasy to iodine with resulting œdema of the larynx and hypersensitiveness to cocaine have been recorded, and in some instances symptoms of asphyxia have been noted which have, however, rapidly disappeared.

REFERENCES

1. Carson.
"*Essays, Physiological and Practical,*" 1822. Wright, Liverpool.
2. Cayley.
Clin. Soc. Trans., 1885, xviii., 278.
3. Forlanini.
Gazz. d. Osp., 1882, iii., 537, etc.

4. Lillingston.
Practitioner, 1913, xc., 129.
5. Beaumont.
Proc. Roy. Soc. Med., 1924, xvii., 37.
6. Burrell and Garden.
Lancet, 1922, ii., 861.
7. Burrell.
Brit. Med. Journ., 1924, i., 368.
8. Parker.
Lancet, 1882, i., 689.
9. Chandler.
Lancet, 1920, i., 764.
10. Burrell.
Lancet, 1923, ii., 790.
11. Jackson.
Amer. Journ. of Roentgenol., 1918, v., 454.
12. Clerf.
Surg. Clin. of N. Amer., 1924, iv., 104.
13. Lynah and Stewart.
Annals of Surg., 1921, lxxiii., 362.
14. Sicard and Forestier.
Bull. et Mém. d.l. Soc. méd. d. Hôp. d. Paris, 1922, xlvi., 463.
15. Sergent and Cottenot.
Journ. de Radiol., 1923, vii., 441.
16. Armand-Delille, Duhamel and Marty.
Presse med. (Paris), 1924, xxxii., 421.
17. Armand-Delille and Gelston.
Amer. Journ of Dis. of Children, 1924, xxviii., 527.
18. Armand-Delille and Moncrieff.
Brit. Med. Journ., 1924, ii., 7.

CHAPTER X

CUTANEOUS PROTEIN TESTS AND DESENSITISATION

The determination of the local reaction of an individual to various protein substances applied to a cutaneous abrasion is of value in investigating the etiology of such diseases as hay fever, asthma and urticaria.

This method has only been practised on an extensive scale during the past few years, but as far back as 1860 Hyde Salter (1), in the first edition of his work on asthma, pointed out that, in his own case, he was subject to three different varieties of asthma. These he calls common asthma, hay asthma and cat asthma. An attack of the latter was induced by the presence of cats, and he makes the interesting observation that "the wound from a claw, whatever be its form, is always surrounded by a white, hard elevation or wheal, very much resembling the appearance consequent on the sting of a nettle."

Hay Fever.—In 1903, Dunbar (2) demonstrated that the skin of a hay fever patient is sensitive to pollen toxin, whereas this is not the case with a normal individual. In England hay fever is usually due to the Timothy grass pollen, although tree pollens may, in some instances, be responsible. The nature of the pollen to which a sufferer from hay fever is susceptible should be determined during the winter; prophylactic inoculation can then be carried out before the next hay fever season commences.

The proteins can be obtained in solution in various strengths, and "pollaccine" is available for testing the cutaneous reactions.

Cutaneous Tests.—The requirements for the test are as follows : Pollaccine, scalpel or needle, spirit lamp, ether or spirit. " Pollaccine " contains pollen toxin and 0·5% carbolic acid. It is supplied in capillary glass tubes containing a varying number of units of pollen toxin, as 5, 15, 50, 150, 500, 1,500 and 5,000 units. The unit of pollen toxin is that quantity which can be extracted from one thousandth part of a milligram of phleum pollen.

The arm is cleaned and scratched with a needle as for the asthma cutaneous tests (see p. 314). The contents of the tube containing the weakest solution are then ejected, by means of a rubber teat, over the abrasion and rubbed in with the needle. If the reaction be positive an urticarial wheal appears in about a quarter of an hour, surrounded by a red areola. It is usually convenient to apply the four weakest dilutions to different abrasions at the same time, and if no reaction occur the three stronger dilutions can be tested on fresh sites. If this precaution be not taken, and all the dilutions be tested simultaneously, a very marked local reaction may occur on the arm if the patient be very susceptible.

Instead of using the skin, the conjunctiva may be employed in the test, as was shown by Dunbar (2) in 1903.

In this case the pollen toxin containing no carbolic acid preservative is used. A positive reaction consists in a slight reddening of the eye and takes place in three minutes. In general it will be found that the eye will react to a dilution of toxin of one-third the strength required to produce a dermal reaction.

Prophylactic Inoculation

This is carried out during the winter and spring. Dunbar (2), in 1903, found that injection of pollen toxin into animals gives rise to an antitoxin, and Noon (3), in 1911, worked out the technique of active immunisation by the injection of

toxin in man. Therapeutic immunisation was further investigated by Freeman (4).

The amount of toxin to be injected is determined by the strength required to produce a cutaneous or ophthalmic reaction. Thus, if the skin react to 15 units the eye will give a positive result with 5 units, and one-third of the latter quantity constitutes the prophylactic dose for subcutaneous injection. This injection should be repeated every ten to fourteen days for several months.

Asthma.—The cutaneous reactions in asthma have been investigated in America especially by Chandler Walker (5) and in England by Coke (6). The proteins are obtainable either as dry powders, solutions or pastes.

The paste preparations are put up in small collapsible tubes and have certain advantages. They are easy to apply and relatively stable. Thus, whereas the solutions, unless freshly prepared, are liable to lose their activity, it is claimed that the pastes remain potent for at least six months and usually for considerably longer. The solutions and pastes may be obtained containing only one protein in each preparation, or in group form wherein several allied proteins are mixed. In the latter case, if a positive reaction be obtained with a group preparation, the individual proteins must subsequently be tested separately in order to determine which are responsible for the reaction.

More reliable results are obtained by employing the individual proteins, as the substance to which the asthmatic is sensitive may only be present in minute quantities in the mixture.

The obvious drawback to the individual method lies in the large number of proteins which may have to be tested. A study of the table given below will enable the reader to select and test first those proteins which are most likely to give a reaction in a routine examination. Chandler Walker obtained a positive cutaneous reaction in 48% of 400 cases tested, and Coke shows 52% positive in a series of 350 cases.

A comparison of their results is given in the following table :—

	Chandler Walker (5).	*Coke* (6).
Pollens	23%	11·1%
Animal hair	19·5%	23·7%
Cereals	8·7%	12·9%
Bacteria	8·2%	4·9%
Eggs	3·2%	2·6%
Fish	2·0%	1·4%
Potato	1·7%	1·7%

The skin over the flexor surface of the forearm is cleaned with spirit. If a large series of proteins is to be tested the whole of the arm and forearm will be required, but the skin in the region of the antecubital fossa should be avoided, as it is more sensitive to the protein than other parts. Two or three small scratches are then made with a sharp scalpel or needle sterilised in the flame. They should be sufficiently deep to remove the surface epithelium, but not to draw blood. A little of the paste is taken up with the needle or scalpel and rubbed into the abraded area. The instrument is then wiped clean and resterilised. Similar scratches and inoculations are made in a regular series down the arm, about $1\frac{1}{2}$ inches apart, and, if necessary, two rows can thus be formed. A control with a paste free from protein should also be made. The procedure is the same with powders or solutions, but in the former instance a drop of $\frac{N}{10}$ caustic soda solution is placed on the abrasion with a platinum loop, and with the loop still wet a little of the powder is taken up and mixed with the soda solution on the skin. The solutions are either shaken out from the glass tubes or ejected with a rubber teat.

The Reaction.—In the case of a positive reaction a red area appears at the site of the abrasion, usually within a few minutes. This increases in size and may be accompanied by itching. This is followed by the formation of an urticarial

wheal, which should reach to a diameter of at least 0·5 cm., and has often an irregular outline. The maximum reaction is usually obtained in half an hour, at which time the proteins may be washed off each abrasion with a little cotton wool soaked in saline or water. A fresh piece of cotton wool must be used for each spot, so that they may not be contaminated by other proteins. The reactions are then read.

In some cases a reaction is delayed and the patient should be instructed as to this eventuality, and asked to record on a chart the site at which any reaction may appear within the next twenty-four hours.

Urticaria.—Similar protein cutaneous tests may be carried out in cases of intractable urticaria.

Desensitisation in Asthma and Urticaria

After the discovery of the specific protein or proteins to which a patient gives a dermal reaction, the question of special treatment has to be considered. There are two main methods: Avoidance of the offending proteins; or desensitisation by minute injections of the corresponding proteins.

In the case of articles of food, it is usually easy to omit from the diet any particular substance to which the patient is sensitive. Animals can also generally be avoided, but difficulty arises with certain of their emanations, such as dandruff, which is so universally present in the atmosphere of towns. Patients sensitive to feathers must be careful to avoid pillows, beds and eiderdowns containing feathers. Kapoc (vegetable down) pillows may be used.

In cases of asthma due to horse dandruff the dermal reaction is tested with progressively weaker dilutions of the protein, until the weakest dilution causing a reaction is obtained. The dilutions used are usually 1/1, 1/10, 1/1,000, 1/10,000, 1/100,000 and 1/1,000,000. Desensitisation is attempted by subcutaneous injections of the protein, commencing with 2 m. of a solution one-tenth weaker than the

weakest dilution giving a dermal reaction. The injections are made twice a week, increasing by 1 m. each time until a dose of 10 m. is reached. The next injection is then 2 m. of a solution ten times as strong, and so on, the dose and strength of the injections being gradually increased, and the dermal reactions tested from time to time to make sure that desensitisation is being effected, and that the strength of the solutions injected is not sufficient to cause a cutaneous reaction.

It will be seen that desensitisation by this method is slow, and may take several months. Further, unless the patient is in the country away from horse dandruff during this time successful results are not likely to be obtained.

Coke (6) recommends that desensitisation against specific articles of food may be attempted by giving them in small doses in pill form. Thus a pill containing $\frac{1}{8}$ gr. of pea and crushed haricot bean may be taken three times a day at first, and the amount gradually increased. This applies especially to those cases in which, owing to occupation, it is impossible to avoid coming in contact with the foodstuffs in question. Injection of food proteins has not given satisfactory results.

Certain cases of bronchial asthma, in which the sputum shows some predominating organism, respond well to vaccine treatment. We have obtained the best results in these cases with an autogenous vaccine of *B. Friedländer*, the pneumococcus, the micrococcus catarrhalis, and, in some instances, with the streptococcus. It is advisable to commence the injections with small doses, such as five millions, and gradually to work up to 40 or 50 millions, if no undue reaction ensue.

Non-specific vaccines may also be of value in producing desensitisation in cases of asthma, especially those obtained from the patient's fæces. These usually consist of streptococci and the *B. coli*, and may be given in a mixed vaccine.

Auld (7) recommends the use of peptone as a method of desensitisation common to cases of asthma caused by various

proteins. A 5% solution of Armour's No. 2 peptone may be used. The initial dose is 5 m. of the solution, given slowly intravenously. The injections are repeated twice a week, the dose being increased by 3 m. each time until six injections have been given. The last dose (20 m.) is repeated five or six times. No injections must be given during an attack, and the patient should be in bed and under observation, at any rate for the first few injections. The temperature should be recorded about six hours after the injection, and if it rise over 1° F. the next injection should not be increased. In children Auld (8) recommends that the injection should be made into the spinal muscles, in which there is an extensive venous plexus. Witte's peptone is not suitable, as it gives rise to too great a reaction, owing to the proportion of primary to secondary proteoses in it being relatively too great.

Auld (9) has further recommended the use of serum peptone for cases of asthma which have not responded satisfactorily to the administration of peptone. The latest preparation is known as " new serum peptone," and is prepared by Auld (10) as follows : Five hours after the last meal, 2 to 3 ounces of blood are run from the patient's vein into a sterile glass tube. It is allowed to stand at room temperature, and next day the serum is pipetted off. One volume of agar solution is then shaken up with 5 volumes of serum, and incubated at 37° C. for an hour. The agar solution is prepared by mixing one part agar with a thousand parts water, which is then boiled well and cooled. Armour's No. 2 peptone powder is now added to the serum agar mixture, in strengths of 4, 5 or 6%, as required to suit the patient, and the mixture is incubated for an hour. The peptone thus dissolves and 0·5% phenol is added. The initial dose is 1·5 c.c. given intravenously, and this is gradually increased to 3 or 4 c.c., the injections being repeated every three, four or five days. The weaker peptone solutions should be used for delicate patients having frequent attacks. The author states that

the solutions must be very carefully prepared and administered. This method is still on trial.

Oral administration of peptone has also been tried, in doses of 3·5 to 7 gr., in capsules three times a day, half an hour before meals, as recommended by Widal (11).

Although the method of testing the dermal reactions with subsequent attempts at desensitisation is at times followed by brilliant results, it must be admitted that the failures are numerous. One of the great difficulties is that it is almost impossible to test the reaction to all the proteins to which a patient might be sensitive. Thus Freeman (12) states that "only 1% or less of the possible proteins are available for testing." The method is, therefore, one which always involves the outlay of a great amount of time, and which frequently brings no relief to the patient.

REFERENCES

1. Hyde Salter.
"*Asthma*," 1860, 342. Churchill, London.
2. Dunbar.
"*Zur Ursache und specifischen Heilung des Heufieber*," 1903. München.
3. Noon.
Lancet, 1911, i., 1572.
4. Freeman.
Lancet, 1911, ii., 814.
5. Chandler Walker.
Boston Med. and Surg. Journ., 1918, clxxix., 288.
6. Coke.
"*Asthma*," 1923. Wright & Sons, Bristol.
7. Auld.
Brit. Med. Journ., 1918, ii., 49, and *ibid.*, 1921, i., 696.
8. Auld.
Brit. Med. Journ., 1920, i., 567.
9. Auld.
Brit. Med. Journ., 1925, i., 448.
10. Auld.
Brit. Med. Journ., 1926, ii., 732.
11. Widal.
Lancet, 1922, i., 1112.
12. Freeman.
Brit. Med. Journ., 1924, i., 523.

CHAPTER XI

THE DETERMINATION OF THE SUSCEPTIBILITY TO DIPHTHERIA ; THE TREATMENT OF CARRIERS, AND IMMUNISATION AGAINST THE DISEASE

The Schick Reaction

This test was devised in 1913 by B. Schick (1) with the object of determining the susceptibility of individuals to diphtheria.

The requirements for the test are as follows :—

Diphtheria Toxin.—This must be potent and properly diluted, so that 1 c.c. contains 1/10 of a minimum lethal dose (M.L.D.). The M.L.D. of diphtheria toxin is the minimum amount which will kill a guinea-pig weighing 250 g. in four days. The potency of the toxin is an essential factor in the reliability of the test. The diluted toxin is not stable ; therefore it should be kept in an ice chest, and used at the latest within a week, and preferably within twenty-four hours of dilution.

Control diphtheria toxin, which has been heated to 75° C. for ten minutes.

A syringe, graduated in tenths of a c.c., with a fine, sharp, closely-fitting needle (No. 1 dental needle).

Alcohol, to clean the arm.

The Test.—The flexor surface of the forearm is cleaned with alcohol. A site near the widest part of the forearm is chosen. The syringe is filled with the toxin, and the needle introduced intradermally for about half an inch, with the bevelled opening at the point of the needle near the surface, and just showing through the superficial layers of the skin.

This is best done by holding the back of the patient's arm with the left hand, and putting the skin on the stretch by backward pressure with the fingers and thumb. The syringe should be held almost parallel with the arm. Two-tenths of a c.c. of the toxin (1/50 M.L.D.) are injected, thus forming a small wheal which persists for a few minutes to half an hour. A certain amount of force is required for this injection, and it is therefore essential that the needle and syringe fit accurately to prevent leakage. The site of the injection may be sealed with gauze and collodion to prevent the fluid leaking out, and the seal removed in two hours (Dickinson) (2), but this is not necessary if the needle be withdrawn slowly. The syringe is now washed out, and an equal amount of the control heated toxin is injected in a similar manner into the opposite arm.

The resulting reactions are read after twenty-four hours, seventy-two hours, and ten days. Four possible reactions may occur :—

Positive Reaction.—This indicates that the individual is susceptible to diphtheria and that his blood contains little or no antibody. After twenty-four to thirty-six hours a dark red raised swelling, about half to one inch in diameter, round or oval in shape, is seen at the site of the injection of the toxin. There is little induration. The swelling reaches its maximum about the third day and then gradually fades away during the course of a week. The skin becomes a little pigmented, and some degree of scaling may be seen, lasting to the tenth day. The control arm shows no reaction.

Negative Reaction.—This is interpreted as showing that the individual contains sufficient antibody in the blood to render him immune to diphtheria. No swelling appears after the injection, and if any redness be seen it is present in the form of a line along the needle track. The control arm also shows no reaction.

Pseudo-reaction.—Here a false reaction occurs, not due to

the toxin, but to the presence of bacterial proteins, or other substances not yet identified, which are present in the toxin preparation, and are known as "pseudo-constituents." It indicates that antibodies are present in the blood, and that the individual is immune to diphtheria. Dudley (3) found that pseudo-reactions were more common amongst those boys he examined who had previously given positive reactions, but subsequently gave negative ones, and who had, therefore, developed immunity themselves. He concludes that "the pseudo-reaction is probably due to fairly recent sensitisation of the skin by the proteins of the diphtheria bacillus." The characteristics of this reaction are the rapid appearance of a bright red swelling without any sharp edges, and often somewhat indurated and urticarial. It is seen usually on the first day and fades away in two or three days, leaving behind little or no pigmentation and no desquamation. It occurs on both arms, and the reaction is no greater on the arm injected with unheated toxin than on the control. "Giant" pseudo-reactions have been observed by several workers. These extend over a large area of the arms, but rapidly subside after reaching a maximum in about forty-eight hours. Pseudo-reactions are more common in adults than in children.

Combined Reaction.—This is evidence that the individual is susceptible to diphtheria, and also reacts to the "pseudo-constituents" of the test toxin. According to Dudley (3), it probably represents a transitional stage between susceptibility and immunity. A reaction is obtained in both arms, that on the control side being a pseudo-reaction, whereas that on the test arm is a combination of a pseudo-reaction and a positive reaction. It therefore lasts longer and gives rise to some pigmentation and desquamation.

The Value of the Test.—The reliability of the test, as stated above, depends not only on the technique employed, but also upon the potency of the toxin used in its performance. Examples of differences in reaction, when toxin pre-

pared by different manufacturers is used, are given by Peters (4).

Generally speaking, it is found that a positive reaction is obtained in about 15% of children under three months and in 60% during the first year of life. The greatest number of positive results are obtained between the ages of one and two years, when about 70% react. About 20% of adults show susceptibility to diphtheria; thus Zingher (5), testing 1,483 healthy recruits in America, found 17·6% gave a positive reaction. Peters (4) quotes examples of cases which, although immune according to the Schick reaction, within a short time subsequently contracted diphtheria with virulent organisms in the throat. O'Brien and others (6) have recently reviewed the occurrence of diphtheria in Schick-negative reactors. Only 18 cases of diphtheria were met with in a population of over 20,000 Schick-negative individuals during a period of six years, and the disease was invariably slight, with little or no toxæmia.

It is claimed that the reaction is, to a certain degree, quantitative, a positive result indicating that the blood contains less than 1/30 unit of antitoxin per c.c., whereas a negative reaction shows that a greater amount of antitoxin than this is present. According to Dudley (3), a negative Schick reaction is always given by carriers of virulent diphtheria bacilli, but carriers of a virulent bacilli may give either a positive or a negative reaction. If virulent bacilli be found in the throat of a person giving a positive Schick reaction, it is an indication that diphtheria is in the incubation stage and that clinical diphtheria will be apparent in a few days (Okell, Eagleton and O'Brien (7)).

The Treatment of Carriers

It is often extremely difficult to rid the throat of diphtheria bacilli in the case of carriers, but if the organisms be virulent,

as judged by their toxicity to a guinea-pig, the carriers must be isolated until free.

Various methods, such as the use of gargles and throat sprays, have been employed, generally ineffectually, and in some cases the tonsils have been removed. Good results are claimed by the injections of vaccines of diphtheria bacilli, either stock cultures (Brownlie (8)), or autogenous ones (Eyre (9)), in doses of five to ten millions at intervals of five to seven days. One or two such injections usually suffice. In dealing with large numbers the use of autogenous vaccines is obviously somewhat impracticable. Reith Fraser and Duncan (10) recommend detoxicated vaccines in large doses as the best method of curing persistent carriers, 20 millions to 350,000 millions of the organism being given subcutaneously.

Immunisation

Passive immunisation, by means of diphtheria antitoxin, is the only method available for treating active infection with the diphtheria bacillus, resulting in clinical diphtheria. In adults, 8,000 units should be given subcutaneously, followed by subsequent injections, the amount of which is determined by their effect upon the local lesion and the general disturbance. Schick (11) recommends that the amount of antitoxin given in a case of average severity should be 100 units per kg. of body weight, and that the maximum amount should be 500 units per kg. of body weight. He states that larger doses than this have no greater beneficial effect. Intramuscular and intravenous injections produce more rapid results, and should be used in very severe cases. The serum treatment produces only a short-lived immunity, which, as judged by the Schick reaction, persists on the average for about three to ten weeks. Dudley (3) found that in boys who had suffered from diphtheria and had been treated with antitoxin, subsequent examination some months later showed a negative Schick reaction in a high percentage of

cases. This is considered due to the fact that the boys were subsequently exposed at school to infection with the diphtheria bacillus. The attack of diphtheria acted as a " primary stimulus " sensitising the patient, and reinfection did not produce a second attack of diphtheria, but acted as a " secondary stimulus " and produced sufficient antitoxin to give rise to a negative Schick reaction.

Active Immunisation.—Individuals not suffering from diphtheria, but susceptible, according to the Schick reaction, may be desensitised by the method introduced by von Behring (12), which has been extensively employed by Park and Zingher (13) in New York. This consists in the subcutaneous injection, usually over the deltoid region, of a mixture of diphtheria toxin and antitoxin or toxoid antitoxin, known as " diphtheria prophylactic " (B. W. & Co.). Toxoid is toxin which has been treated with formalin and heated. It is atoxic, but antigenic. One c.c. of the mixture can be given to a child. One-half c.c. is usually given to a child under one year for the first injection, but in some cases severe reactions occur, and it is safer to begin with an injection of $\frac{1}{5}$ or even $\frac{1}{20}$ c.c., followed by a second injection of $\frac{1}{4}$ c.c., and a third dose of 1 c.c. In all, three injections are given at weekly intervals. The reaction is usually considerably greater in adults than in young children, especially in those adults who give a pseudo-reaction to diphtheria toxin. For this reason the initial dose should not exceed $\frac{1}{10}$ c.c. If this be not followed by a local or general reaction, 1 c.c. can be injected two days later, followed by two more injections of 1 c.c. at intervals of a week. The toxin-antitoxin mixture should be used within a few weeks of its preparation, but if kept, the toxin disappears before the antitoxin. Frozen toxin-antitoxin has, however, been shown to be dangerous, the antitoxin being destroyed by the intense cold and the toxin remaining potent (14). O'Brien (15) believes that the freezing and thawing causes a local concentration of phenol to occur, and so dissociation and destruction of the antitoxin

takes place. The immunity takes from one to three months to develop, and the duration of the protection afforded is not yet known, although it seems probable that it may persist for long periods as the result of subsequent production of antitoxin in the body aroused by the stimulus of the injections. Thus Parish and Okell (16) examined 440 children who were previously Schick-positive and had been made Schick-negative by immunisation. They were re-tested one to seven years later, and 95% remained negative reactors. Benson (17), in the Edinburgh City Fever Hospital, has demonstrated the practical value of prophylactic immunisation of nurses. Two hundred and nineteen Schick-positive reactors were immunised, and the incidence of diphtheria amongst the nursing staff was reduced by at least 85%. Further, 95% out of 143 immunised nurses, subsequently re-tested, were found to remain negative reactors at the end of a year.

If it be desired to produce a rapid, although temporary immunity, antidiphtheritic serum must be employed, the dose being 50 units per kg. of body weight.

REFERENCES

1. Schick.
Münch. med. Woch., 1913, ix., 2608.
2. Dickinson.
Lancet, 1922, i., 312.
3. Dudley.
Med. Research Council Spec. Report Series, 1923, No. 75.
4. Peters.
Lancet, 1923, ii., 784.
5. Zingher.
Journ. Amer. Med. Assocn., 1918, lxx., 228.
6. O'Brien, Okell and Parish.
Lancet, 1929, i., 149.
7. Okell, Eagleton and O'Brien.
Lancet, 1924, i., 800.
8. Brownlie.
Lancet, 1920, i., 706.
9. Eyre.
Lancet, 1920, i., 838.

10. Fraser and Duncan.
Lancet, 1920, ii., 994.
11. Schick.
Zeit. f. d. Ges. Exper. Med., 1914, iv., 83.
12. von Behring.
Deutsch. med. Woch., 1913, xxxix., 873.
13. Park and Zingher.
Journ. Amer. Med. Assocn., 1915, lxv., 2216
14. *Lancet*, 1924, i., 505.
15. O'Brien.
Lancet, 1925, i., 102
16. Parish and Okell.
Lancet, 1928, ii., 322.
17. Benson.
Edin. Med. Journ., 1928, xxxv., 617.

CHAPTER XII

THE DETERMINATION OF THE SUSCEPTIBILITY TO SCARLET FEVER. IMMUNISATION AGAINST THE DISEASE

The Dick Reaction

In 1924 George and Gladys Dick (1) introduced a skin test for determining susceptibility to scarlet fever, analogous to the Schick reaction for diphtheria described in the previous chapter.

This involved, first of all, the isolation of what is taken to be the causative organism of scarlet fever, and then the preparation of a toxin from it. The Dicks had previously experimented with various forms of hæmolytic streptococci, but failed to produce experimental scarlet fever. Thus, in 1921 (2), they tried eighteen species of streptococci taken from the throats of scarlet fever patients within seventy-two hours of the onset of the disease. They swabbed the throats of thirty volunteers with cultures of the streptococci, with negative results in twenty-three instances. Seven of the volunteers developed a sore throat, fever and leucocytosis, but in no case was a rash produced.

In 1923 the Dicks (3) succeeded in producing experimental scarlet fever in man, using a hæmolytic streptococcus isolated from the finger of a scarlet fever patient. The organism was obtained from the pus on the second day of the disease, the sore finger having first been noticed two days before the onset of the scarlet fever. A four days' old culture of the hæmolytic streptococcus, grown on sheep's blood agar, was used to swab the throats of five volunteers, and one developed typical scarlet fever with sore throat, pyrexia and rash. The

Dicks (4) state that the hæmolytic streptococci causing scarlet fever fall into two groups, as judged by their power of fermenting mannite, and that both produce toxins of scarlet fever and have their own agglutinating reactions.

It is probable that the specific hæmolytic streptococcus which causes scarlet fever is widely distributed, for it has been isolated by various observers from such sources as a wound, a normal throat, and the throat of a patient suffering from measles.

The Toxin.—This was first prepared by the Dicks (1) from the hæmolytic streptococcus isolated from the finger of the scarlet fever patient. The filtrate from this culture did not produce clinical scarlet fever on injection, but contained the toxin required for testing the susceptibility of individuals to the disease. The toxin is used in dilutions of 1/500 to 1/2,000, in normal saline. The actual dilution required depends upon the strength of the toxin, which in turn is probably dependent on the organism used for its production. Thus Ker and his co-workers (5) in Scotland, using hæmolytic streptococci obtained from the throats of early cases of scarlet fever, considered that their toxin was weaker than that in use in America.

There is no laboratory standard for the strength of the toxin as is the case with diphtheria tóxin, for the scarlet fever toxin is not pathogenic to laboratory animals. The only method at present available for determining its strength is to compare it with a known standard toxin as judged by the reaction given when it is injected intracutaneously. The injection is made into the skin of the forearm of a susceptible person, as in the Dick test.

The diluted scarlet fever toxin is comparatively stable and so does not require dilution shortly before injection, as does the diphtheria toxin employed in the Schick reaction.

The Control Toxin.—The toxin is boiled in a water bath for one hour, either when diluted to 1/100 or 1/1,000. It is

thus seen to be more resistant to heat than is the diphtheria toxin.

The Test.—This is performed in exactly the same way as is the Schick test described in the previous chapter. One-tenth of a c.c. of the toxin is injected intradermally into the flexor surface of one forearm (usually the right) and an equal amount of the control heated toxin is injected in a similar manner into the opposite arm. The reactions are read after four, twelve, twenty-four and forty-eight hours. The twenty-four hour reading is usually the most valuable. As in the Schick test, four possible reactions may occur :—

Positive Reaction.—This indicates that the individual is susceptible to scarlet fever, and has little or no antitoxin in his blood. The reaction is first seen in four to six hours and reaches its maximum in twenty-four hours. The Dicks (1) distinguish three grades of positive reaction at twenty-four hours, which they describe thus :

(1) *Slightly positive.* A faint red area less than 2 cm. in diameter. There is no swelling and no tenderness.

(2) *Positive.* A red area 1·5–3 cm. in diameter, accompanied by some swelling and tenderness.

(3) *Strongly positive.* The red area extends for 3–7 cm. in diameter and is accompanied by tenderness and a swelling with a sharply raised edge which reaches beyond the red area.

The positive reactions all fade after forty-eight hours—a fine desquamation occurs on the seventh to tenth day, but there is usually no pigmentation. In all these degrees the control arm shows no reaction.

Negative Reaction.—This is a sign that the negative reactor is immune to scarlet fever, owing to the presence of antitoxins in the blood. There is usually no alteration in the appearance of the skin at the site of the inoculation in either arm, but at times a faint pink streak may be seen along the needle track.

Pseudo-reaction.—This resembles the pseudo-reaction

described in the Schick test. It is considered to be due to certain proteins in the test fluids and not to toxin. It occurs on both arms as an area of redness, with or without swelling. Both arms present identical appearances. Marked, moderate and slight pseudo-reactions are described by Zingher (6) and classified by him as pseudo 3, pseudo 2 and pseudo 1 respectively. The reaction disappears more rapidly than the true positive reaction.

Its significance is that the pseudo-reactor is immune to scarlet fever, owing to the presence of antitoxins in the blood.

Pseudo-reactions appear to be more common after active immunisation against scarlet fever, as described later (Zingher (6)).

Combined Reaction.—This also corresponds with the similar reaction in the Schick test, and has a like significance. Reactions appear both on the test and on the control arm ; the former is, however, more marked, being a combination of a positive and a pseudo-reaction, the control arm showing only a pseudo-reaction. Combined reactors are susceptible to scarlet fever.

The Value of the Test.—Here again the results obtained depend not only on the accuracy of the technique, but also on the potency of the toxin employed. Ker (5), using a toxin prepared from hæmolytic streptococci, obtained from the throats of early cases of scarlet fever, found a lower percentage of positive results than did Zingher (6). Thus, during the early stages of scarlet fever, Zingher found that of 141 cases 100% gave a positive result during the first five days of the illness, whereas Ker, in twenty-three cases tested during the first three days of the disease, found only 73·9% positive. Not all of Zingher's cases, however, gave a negative reaction during convalescence, but in 170 cases tested, 93% were positive early in the disease and negative during convalescence. Zingher states that a definite negative reaction during the early stage of suspected scarlet fever is strong evidence against such a diagnosis, although in some

cases, where there is a diffuse rash on the flexor aspect of the arm, a slightly positive reaction may be overlooked. A marked positive reaction after the third day of an illness is also evidence against the presence of scarlet fever. As regards the susceptibility in general of the population towards scarlet fever, as judged by the Dick reaction, the figures of Zingher (6) and of Ker (5) agree more closely. A positive reaction, according to Zingher, is given in 44·8% of infants under six months of age. The greatest susceptibility is between the ages of one and two years, when 70·7% are positive. After this age susceptibility gradually falls until after twenty years only 17·9% give a positive reaction. These figures are based upon observations made in a series of 4,570 cases. Okell and Parish (7), however, found a positive reaction in 74% of 100 normal medical students, and Benson (8) showed that 33% of nurses in a fever hospital were positive reactors.

Immunisation

Passive Immunisation.—The Dicks (9) immunised a horse by injecting subcutaneously the sterile filtrate obtained from broth cultures of the hæmolytic streptococcus which they had previously shown was capable of producing scarlet fever in volunteers. They were thereby able to produce an antiserum which could be concentrated without losing its specific properties. This antitoxin will neutralise the scarlet fever toxin as judged by cutaneous tests, but no method of standardising it through animals has yet been found, as the toxin is not pathogenic to them.

This anti-scarlatinal serum should be used in early stages of the disease, *i.e.*, in those cases in which hæmolytic streptococci are obtained from the throat and which also give a positive Dick test. In such cases there is not time to produce active immunity by means of injecting scarlet fever toxin (Dick and Dick (10)). The dose of concentrated scarlet fever

streptococcus antitoxin (P. D. & Co.) recommended is 10 c.c. for an adult, injected intramuscularly into the gluteal region. This amount is able to neutralise about 500,000 skin-test doses of scarlet fever toxin. The unit of antitoxin is that amount which will neutralise 100 skin-test doses of toxin, as judged on injection into a strongly Dick-positive reactor. A second injection may be given after an interval of twenty-four to thirty-six hours, if the temperature and pulse do not show a definite improvement. Before giving an injection it is advisable to determine whether the patient is sensitive to the proteins of horse serum, by injecting intradermally 0·2 c.c. of the antitoxin diluted 1 in 10 with normal saline. If the patient be sensitive an urticarial wheal surrounded by an erythematous zone will appear, taking an hour or more to fade away. Preliminary desensitisation must then be effected by subcutaneous injections every half hour of the serum, beginning with $\frac{1}{3}$ m. of the serum (diluted with saline and doubling the dose at each injection until 1 c.c. has been given). The remainder of the serum may then be given. If serum from a scarlet fever convalescent patient be available, it is preferable to the antiserum produced from the horse, as it avoids sensitising the patient to horse serum. The serum appears most valuable in the treatment of toxic cases of scarlet fever (Craig (11)), and a more rapid effect is obtained by intravenous injection. Banks and Mackenzie (12), in a series of over 400 cases of scarlet fever, largely of a mild type, treated intravenously, found a very beneficial effect from the serum. Convalescence was rapidly established and the average stay in hospital was reduced to just over two weeks. They injected 20 c.c. of the serum for an adult and 10 c.c. for a child intravenously, undiluted but warmed to body temperature. In 60% of the cases there was a rigor and rise of temperature within three-quarters of an hour after the injection.

The concentrated streptococcus antitoxin can also be employed to produce a rapid immunity in contacts of a case

of scarlet fever. The Dick-positive reactors are given 5 c.c. of the antitoxin intramuscularly the day after testing. The immunity thus produced lasts from ten to fourteen days, as shown by subsequent skin tests.

Active Immunisation.—The Dicks (13) showed that the injection of suitable quantities of scarlet fever toxin (scarlet fever prophylactic) into persons who are susceptible, as shown by the skin test, may give rise to what appears to be a mild attack of scarlet fever. This is evidenced by a skin rash, nausea, vomiting, pyrexia and malaise. The symptoms are short-lived, appearing a few hours after injection and disappearing usually in forty-eight hours. It was found that after this constitutional reaction the skin test becomes negative.

In order to avoid a marked reaction, Zingher (6) recommends the following procedure in active immunisation. Three injections of the scarlet fever toxin are given once a week, either subcutaneously or better intramuscularly. The dosage is as follows :—

For Children under Twelve Years of Age.—First injection, 100 skin-test doses of toxin. Second and third injections, 250 skin-test doses each.

For Persons over Twelve Years of Age.—First injection 100, second injection 250, and third injection, 500 skin-test doses.

For Adults.—One thousand doses may be given for the third injection. The scarlet fever prophylactic (B. W. and Co.) is put up in two strengths : "A" contains 2,500 skin-test doses, and "C" 20,000 skin-test doses in 1 c.c. The usual reaction to such an injection is purely local, and often there is no reaction with the second and third injections. In some cases a sore throat and scarlatiniform rash have been noted. Attempts are being made to produce a toxoid which, while immunising, will produce no local reaction. The skin test must become negative before immunisation can be considered complete. Benson (8) has also practised active immunisation amongst the nursing staff in a Fever Hospital.

He gave an initial injection of 500 skin-test doses of scarlet fever toxin (scarlet fever prophylactic) followed on the eighth day by 1,000 and on the 15th day by 5,000, and on the twenty-ninth day by 20,000 skin-test doses of the toxin. The majority became Dick-negative reactors within four weeks after the last injection.

The immunity produced by this massive inoculation is considered to last for about two years.

REFERENCES

1. Dick and Dick.
Journ. Amer. Med. Assocn., 1924, lxxxii., 265.
2. Dick and Dick.
Journ. Amer. Med. Assocn., 1921, lxxvii., 782.
3. Dick and Dick.
Journ. Amer. Med. Assocn., 1923, lxxxi., 1166.
4. Dick and Dick.
Journ. Amer. Med. Assocn., 1924, lxxxii., 1724.
5. Ker, McCartney and McGarrity.
Lancet, 1925, i., 230.
6. Zingher.
Journ. Amer. Med. Assocn., 1924, lxxxiii., 432.
7. Okell and Parish.
Lancet, 1925, i., 712.
8. Benson.
Edin. Med. Journ., 1928, xxxv., 617.
9. Dick and Dick.
Journ. Amer. Med. Assocn., 1924, lxxxii., 1246.
10. Dick and Dick.
Journ. Amer. Med. Assocn., 1924, lxxxiii., 84.
11. Craig.
Lancet, 1928, ii., 1123.
12. Banks and Mackenzie.
Lancet, 1929, i., 381.
13. Dick and Dick.
Journ. Amer. Med. Assocn., 1924, lxxxii., 544.

CHAPTER XIII

THE NERVOUS SYSTEM

Spinal Cord Compression

In addition to the neurological signs and symptoms found in a case of spinal cord compression, certain special examinations are of value in confirming the diagnosis and further localising the site of the lesion. These include examinations of the cerebro-spinal fluid by cisternal and lumbar puncture, and intrathecal injection of lipiodol.

Combined Cisternal and Lumbar Puncture

Ayer (1) showed that normally the cerebro-spinal fluid obtained either by cisternal or lumbar puncture has the same composition and exhibits similar pressure variations. In spinal cord compression, however, changes in the pressure relations and chemical composition of the fluid above and below the block may occur. Dixon and Halliburton (2) in 1913 first performed direct cisternal puncture through the occipito-atlantoid ligament in the dog. In 1919 Wegeforth, Ayer and Essick (3) demonstrated the method of cisternal puncture in man, and in 1921 Ayer (4) published an account of the pressure and chemical changes in the fluid above and below a spinal block.

The patient lies on the side, with the head flexed, but the spine below is straight and the cervical and lumbar regions are in an absolutely horizontal plane as determined by a spirit level. Two lumbar puncture needles are used (size 18), each needle has a three-way stop-cock, and a glass manometer (bore 1·5 mm.) is attached to the upright arm.

Cisternal puncture is performed by placing the thumb of the left hand on the spine of the axis vertebra, and inserting the needle in the mid-line just above the thumb. It is pushed in through the skin, forwards and upwards, in a line with the plane joining the external auditory meatus and the glabella. The occipito-atlantoid ligament is encountered at a depth which varies from 3 to 5 cm. The dura mater and arachnoid are pierced immediately after passing through this ligament, and the cistern is thus entered. The antero-posterior diameter of the cistern at this level is between 1·5 and 2 cm. The needle is then placed in communication with the manometer and the pressure recorded.

A lumbar puncture is next performed and the pressure there similarly registered. Normally the pressures at both ends are seen to vary equally with the heart-beat, with respiration, on coughing or sneezing, or on compression of the jugular veins. Queckenstedt (5) showed in 1916 that compression of the veins of the neck causes a rise in tension of the cerebro-spinal fluid on lumbar puncture.

By observations on the manometric readings of a cistern and lumbar puncture hydrodynamic evidence of spinal block may be obtained. Thus, normally, if 5 c.c. of cerebro-spinal fluid be run out from the lumbar puncture needle the cisternal pressure will fall 30 to 40 mm. If a little fluid be run out from the cisternal needle the lumbar pressure will fall correspondingly. If there be spinal block, however, running out fluid from the lumbar puncture will not cause a corresponding fall in the cisternal pressure, and gentle pressure on the jugular veins will increase the cisternal pressure without affecting the lumbar pressure. These changes are exemplified in the table on p. 337.

The chemical changes in the cisternal and lumbar fluid are also of diagnostic value and constitute what is known as *the loculation syndrome*. The fluid stagnates below an obstruction and the following changes are found: 1. Increase of protein up to 3 or 4% (normal is 0·02%). 2. Xan-

thochromia. The fluid has a yellowish tinge due to extravasated blood pigment from the congested meningeal vessels. 3. Spontaneous clotting, owing to increase of fibrinogen.

The loculation syndrome is sometimes known as *Froin's syndrome*, but Froin (6) described a pleocytosis in the fluid as a characteristic feature, rather than an increase in the protein. Pleocytosis has subsequently been shown rarely to occur.

The hydrodynamic evidences of spinal block, when present, are of greater value than the chemical changes in the fluid. Thus an increase in the protein of the lumbar fluid has been noted in such conditions as acute anterior poliomyelitis and disseminated sclerosis. On the other hand, cases have been recorded by Ayer (4) of a vertebral myeloma and a subdural neurofibroma in which there was no obstruction to the cerebro-spinal fluid and yet the lumbar fluid showed considerably more protein than the cisternal. Further, in some cases a tumour may be present and yet no change be found in the composition or pressure relationships of the cisternal and lumbar fluids.

In the following table from Ayer the typical changes in spinal cord compression, as seen in a large extradural perineural neurofibroma, are recorded :—

	Cistern.	*Lumbar.*
Initial pressure	190 mm.	150 mm.
After withdrawal 5 c.c. from lumbar .	180 mm.	60 mm.
,, ,, 5 c.c. from cistern .	160 mm.	60 mm.
,, ,, 5 c.c. more from lumbar	165 mm.	0 mm.
,, ,, 5 c.c. more from cistern	145 mm.	0 mm.
Pulse oscillations	Just visible.	Just visible.
Respiratory oscillations. . . .	5 mm.	30 mm.
Rise of pressure on compression of jugular veins	300 mm.	No change.
Cells per c.mm.	0	2
Protein per 100 c.c.	33 mg.	267 mg.
Globulin	0	+
Colloidal gold	00,00,00,00,00	00,11,10,00,00

Lipiodol in Spinal Cord Compression

In 1921 Sicard and Forestier (7) injected lipiodol into the cellulo-fatty tissues around the dura mater in the lower lumbar region to determine the permeability of the extra-dural space and to localise a tumour or other cause of spinal compression. They subsequently showed that lipiodol is harmless if injected into the subdural and subarachnoid spaces, and this is the method now employed.

There are two varieties of lipiodol, the ordinary heavy preparation, with a specific gravity of 1350, and a light form, called " lipiodol ascendant," the specific gravity of which is lower than that of the cerebro-spinal fluid. It should therefore pass upwards along the subarachnoid space when injected by lumbar puncture, but it is not usually employed as it tends to stick to the meninges, and is not so opaque to X-rays as is the heavy variety.

The Indications.—The method is available for the localisation of spinal cord compression, whether due to intra- or extra-dural causes. These may be such conditions as fracture, dislocation of the spine, spinal caries, new growths of the vertebræ, new growths of the extra-dural space, meninges, nerve roots or spinal cord, inflammatory thickenings or cicatrisations resulting from traumatic hæmorrhage, and syphilitic or suppurative meningitis. In fracture-dislocation of the spine or spinal caries, however, a lipiodol injection seems hardly called for, as in the former the diagnosis is usually clear, and in the latter the injection would not affect the diagnosis or the treatment.

In the majority of cases a skilled neurological examination will correctly localise the upper level of the compression, but the actual relation of the segmental level, as judged by sensory changes, to the spines of the vertebræ may be difficult to determine. A lipiodol injection through a lumbar puncture will indicate the lower border of a tumour, which may be important from the point of view of operation.

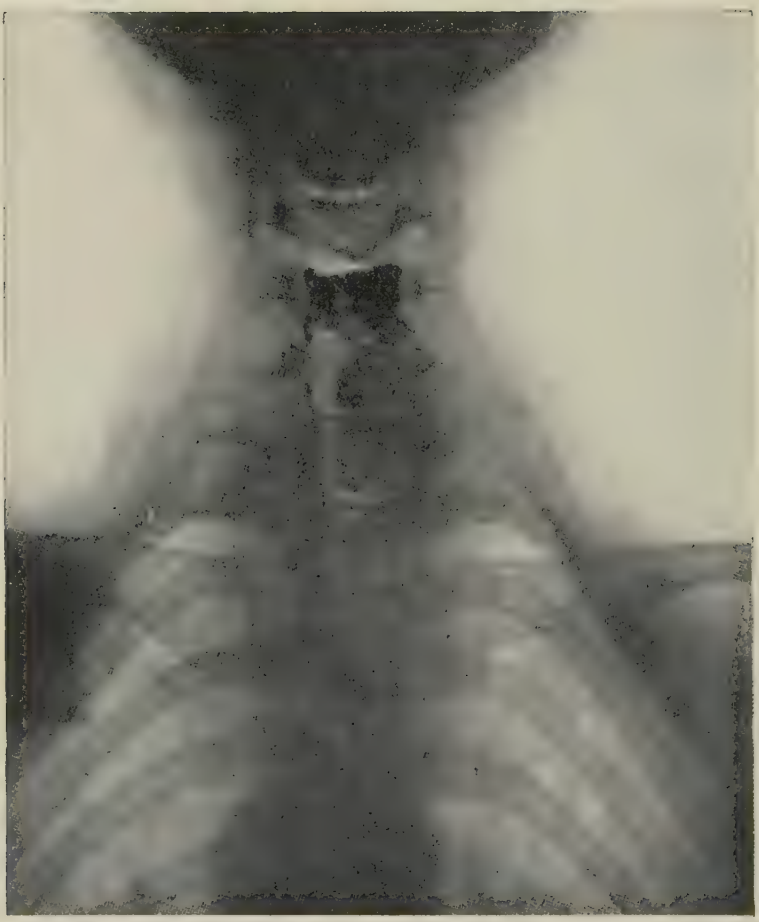


FIG. 43.—Radiogram after lipiodol injection into the cisterna magna, showing obstruction at the level of the sixth cervical vertebra.

[*To face p.* 339.

Difficulties may arise in the interpretation of the results. Thus a *false arrest* of the lipiodol may occur, as it is held up by adhesions to the meninges or nerve roots. In such cases tipping the patient in the other direction will not cause the lipiodol to flow away as it does in a true obstruction. Again a spinal cord tumour may be diagnosed clinically and yet the lipiodol show no evidence of compression, although a tumour is in fact present. This may be due to the presence of a very small tumour or of a large but destructive one. The reverse has also been noted ; thus de Martel (8) records cases in which clinically no tumour was diagnosed, but as the lipiodol indicated the presence of one an operation was performed and no tumour found. Sargent (9), however, records a case of extrathecal endothelioma at the 8th dorsal segment, in which the neurological signs were equivocal, but the lipiodol indicated that cord compression was present. The tumour was removed by operation. In conclusion, Blundell Bankart's opinion is that the neurological localisation of a spinal tumour is usually so accurate that lipiodol injection is rather a surgical luxury in such a case. When the segmental level has been correctly diagnosed there is no difficulty in exposing the required segment or segments by ordinary topographical rules. When there is doubt as to the presence of a tumour or other cause of spinal cord compression the injection of lipiodol may be really useful in determining the presence and level of the compression.

The appearances seen in a case of spinal cord compression at the level of the 6th cervical vertebra, due to a spinal tumour, are shown in Fig. 43.

Apparatus.—Lumbar puncture needle.
Lipiodol syringe to fit above.
Lipiodol.
Minim syringe and fine hypodermic needle.
Sterile novocaine solution, 2%.
Iodine and wool.

The Operation.—*Injection into the Cisterna Magna.*—

The patient may be given a hypodermic injection of morphine, gr. $\frac{1}{4}$, one hour before the operation. He then sits on a chair, with the head flexed ; it should not be depressed too much lest the lipiodol pass straight forward into the cranial cavity. The skin is sterilised with iodine at the back of the neck, the head covered with a sterilised towel, and the skin and subcutaneous tissues anæsthetised with 2% novocaine at the site chosen for puncture. This is a point lying on a line joining the tips of the mastoid processes, in the midline. This point is just above the spine of the axis vertebra. The patient's forehead is supported by the left hand of the operator and the lumbar puncture needle is pushed in at the selected spot, forwards and slightly upwards, in a direction parallel with a line joining the external auditory meatus and the glabella. No bone should be encountered, but should this occur the needle is withdrawn a little and depressed slightly to avoid the occiput. The posterior occipito-atlantoid membrane and dura are thus penetrated, and immediately their resistance is overcome the end of the needle lies in the cisterna magna. This is a very small space, and great care must be exercised not to push the needle into the medulla. The stilette of the lumbar puncture needle is then withdrawn and a few drops of cerebro-spinal fluid should exude. This may not occur and the syringe should then be attached to the needle and gentle suction exerted to see if fluid is obtained, before the needle is pushed in any further, or the medula may be injured. The lipiodol, warmed to body temperature in a water bath, is then injected from the lipiodol syringe attached to the lumbar puncture needle, using 1 to $1\frac{1}{2}$ c.c. The patient is then immediately wheeled in the sitting posture into the X-ray room.

If there be no obstruction the lipiodol will run down to the sacral region in about five minutes ; it may be held up for a little at the level of the 4th dorsal vertebra, where the spinal cord is narrowest. X-ray photographs are taken

immediately after the injection with the head and shoulders raised. If the lipiodol be held up by an obstruction, the photographs are repeated at the end of six hours, twenty-four hours and a week, to see if the level alter.

Injection into the Lumbar Region.—The operation is performed as for lumbar puncture. The patient should lie on his left side, with buttocks raised and head and shoulders depressed. A lumbar puncture is made in the usual way, and 1 c.c. of lipiodol injected into the theca. The patient must then be tilted so that the lipiodol passes towards the head by gravity if the heavy preparation be used, and photographs are taken as in the previous method. The necessary inversion of the patient renders this method more troublesome than that of cisternal puncture, although an operative risk due to lack of skill is not so great.

Difficulties and Some Complications.—A certain amount of manual dexterity is required to perform a cisternal puncture, and it should not therefore be lightly undertaken by the inexperienced owing to the risk of injuring the medulla. Although it is stated that lipiodol is non-irritating to the meninges, yet reactions have been observed in cats by Ayer and Mixter (10), and in man by Armour (11), and by Sharpe and Peterson (12). Such symptoms as headache, nausea and insomnia, pyrexia and an increase of pre-existing root pains have been noted. The cerebro-spinal fluid frequently shows an increased amount of protein and pleocytosis. Further, certain surgeons hesitate to inject into the theca a foreign substance which apparently remains there permanently.

The experience of Sharpe and Peterson (12) was most discouraging. They injected lipiodol in three cases, one by cisternal and two by lumbar puncture. In no instance was the lipiodol absorbed, and in one case, that of the cisternal injection, it became encysted and surrounded by adhesions giving rise to an aggravation of the patient's symptoms, and necessitating subsequent removal by operation. They advise

therefore that lipiodol should only be injected by lumbar puncture, as it will then settle in the caudal region and give rise to no symptoms. Sicard and Forestier (13) state that the lipiodol does not adhere, and can be removed by lumbar puncture if desired after several days.

The Cerebro-spinal Fluid

Although tests under this heading are of a rather specialised type, it was thought advisable to include them in this volume.

Bacteriological investigations and the technique of the Wassermann reaction will not be described.

Examination of the cerebro-spinal fluid is most useful in the diagnosis and treatment of :—

1. Syphilitic lesions of the central nervous system, as dementia paralytica, tabes and cerebro-spinal syphilis.

2. Meningitis.

For a full treatment of this subject, the reader is referred to Nonne's (14) book, "Syphilis und Nervensystem," from which the following account is mainly derived.

Tests in common use :—

The Wassermann reaction.
Lange's colloidal gold reaction.
Globulin and albumin estimations.
Cytological examination and cell counts.
Quantitative estimation of reducing power, reaction, urea, non-protein nitrogen and chloride content.

The Colloidal Gold Reaction (Lange (15))

This test was evolved by Lange from the work of Zsigmondy on protective colloids. He noted that certain colloids, such as gold, could be precipitated from their solution by the addition of electrolytes, such as NaCl, and that certain other colloids were able to protect the gold

against this precipitation. Lange found that whilst normal cerebro-spinal fluid possesses this property, fluid derived from certain pathological subjects lacks this protective action and precipitation occurs on the addition of an electrolyte. On further investigation it was found that precipitation of the gold occurred with fluids derived from cases of general paralysis, general syphilis of the central nervous system, and certain cases of meningitis. Lange also showed that these three lesions could be differentiated, since the maximum precipitation occurred with different dilutions of fluids obtained from the three conditions.

The technique is not an easy one, and many variations of the original Lange process have been introduced. It is proposed here to describe a method taken from a paper by Cruickshank (16).

Method.

Preparation of Gold Chloride Solution.—In order to obtain successful results, great attention must be paid to the cleanliness of the glass, to the manipulations and to the distilled water. It is advisable to clean out all flasks, beakers, etc., with hot nitro-hydrochloric acid prior to use, and to rinse them out with water, distilled twice from glass. All water must be doubly distilled from glass vessels. The following solutions are then made up :—

1% gold chloride.
2% potassium carbonate.
1% pure formalin.

Five hundred c.c. of the doubly distilled water are heated in a beaker to 60° C., and 5 c.c. of the gold chloride and 5 c.c. of the potassium carbonate solution are added. The heat is increased in order to raise the temperature rapidly to 90° C., when heating is discontinued. Five c.c. of formaldehyde solution are added, drop by drop, with constant shaking, until a faint pink colour appears. The formalin is then added in large quantities. In about two minutes the maximum deep red colour will develop. If purplish-red or opaque, the

solution should not be used, although it has been stated that a slight sheen does not matter. The solution should be quite stable. According to Black, Rosenberg and McBride (17), the hydrogen ion concentration of this solution should be tested according to the following method :—

A double row of tubes is set up, each containing 1 c.c. of distilled water. Into the first tube of each row 1 c.c. of $\frac{N}{50}$ acid and 1 c.c. of $\frac{N}{50}$ alkali are added respectively. One c.c. is then removed from the first tube containing the acid, and is added to the next tube, and so on down the row. The same process of dilution is carried out with the alkali. Two drops of 1% alizarin red solution in 50% alcohol and 5 c.c. of gold chloride solution are then added to each tube. A buff colour represents the neutral tube, and from the amount of acid or alkali in it the quantity necessary to neutralise the gold chloride solution can be calculated. This should be done in every case.

Tests of Suitability.—Five c.c. of solution should be completely precipitated by 1·7 c.c. of 1% saline in one hour.

Normal cerebro-spinal fluid should be without action upon the solution.

Fluid from a case of general paralysis should give the typical paretic reaction.

The Test.—Eleven small perfectly clean tubes are set up in a rack, and 0·9 c.c. of 0·4% NaCl solution is added to the first tube, and 0·5 c.c. to the rest. 0·1 c.c. of spinal fluid is added to the first tube, thus making a dilution of 1 in 10. After thorough mixing, 0·5 c.c. is removed and added to the second tube, which in turn is shaken. 0·5 c.c. is removed from this tube to the third, and so on. The resulting dilutions are therefore : 1 in 10, 20, 40, 80, 160, 320, 640, 1,280, 2,560, 5,120 and 10,240. Two and a half c.c. of the gold solution are then added to each tube.

If the colloidal solution be completely broken down, the contents of the tube will be colourless, and the gold will sink to the bottom, whilst if no precipitation has occurred, the original red colour will persist. Between these two extremes there is the following range of colours : colourless, grey-blue, blue, lilac, red-blue and red. It is usual, as Cruickshank (16) states, to express the results numerically according to the following scheme :—

Complete precipitation . .	5
Pale grey-blue	4
Deep blue	3
Reddish-violet	2
Bluish-red	1
Unchanged red	0

There are three types of response :—the paretic, luetic and meningitic. In the paretic response, precipitation only occurs in the tubes with dilutions between 1 in 10 and 1 in 640. In the luetic reaction, the maximum reduction occurs between 1 in 40 and 1 in 80, whilst the meningitic response is mainly between 1 in 320 and 1 in 640.

Expressed according to the numerical scheme :—

Paretic response is . . .	55554331000
Luetic response is . . .	00243110000
Meningitic response is . .	00001344300

These are average findings, and exact adherence to the figures is not necessary.

If blood be added to a normal cerebro-spinal fluid in minute amounts, the response is similar to a meningitic curve, *i.e.*, well to the right of the syphilitic reactions. Hence contamination of the fluid with blood does not impair the diagnosis from the point of view of syphilis, but it must be clearly understood that large quantities of blood will obscure the reaction completely.

Findings.—Lange claimed that the reaction was more sensi-

tive than the Wassermann test, and his views have been confirmed by some workers.

A paretic response is said to be obtained in every case of general paralysis. Tabes dorsalis and cerebro-spinal syphilis give luetic responses. With regard to the interpretation of the meningitic response, observers differ. This appears to be due mainly to leakage of the serum proteins, giving a response similar to that obtained by the addition of minute amounts of blood to normal fluid.

Many writers have pointed out the value of this reaction in syphilis, but the value in meningitis is doubtful. Similar curves are given in tuberculous and purulent meningitis. For a full account the reader is referred to the valuable article by J. Cruickshank (16).

Globulin and Albumin Tests

Normally the fluid contains only a trace of protein. This is increased in meningitis and syphilitic conditions. The increase can be determined by the following tests :—

The Nonne-Apelt reaction (18).

The Lochelongue-Levinson method for estimation.

The Nonne-Apelt Globulin Test.—Add to a carefully prepared saturated solution of ammonium sulphate an equal amount of cerebro-spinal fluid. One c.c. of each is quite sufficient.

Pour one liquid gently on top of the other, and if the globulin be increased there occurs a more or less distinct grey ring at the plane of contact.

The mixture is well shaken, and the result may be read off within three minutes. If distinctly opalescent, or cloudy, it is positive.

It is best to add the cerebro-spinal fluid from a 1 c.c. pipette very slowly to the ammonium sulphate solution, as in this way the grey ring, indicating increased globulin, is more distinct than when one fluid is poured on to the other.

This part of the reaction is known as *phase* 1. The precipitate is filtered off, and a drop of 10% acetic acid is added, and the mixture is boiled. A precipitate forms if albumin be present. This is known as *phase* 2. Nonne states that the second phase is of little diagnostic value, except in a case of non-syphilitic meningitis.

Estimation of Protein in a very Albuminous Fluid.—(*Lochelongue-Levinson*).—This technique is a slight modification of Mestrezat's diaphanometric method. A series of standards is prepared from egg albumin in saline, starting with 0·4% and decreasing to 0·01%. It will be found most convenient to make up the following dilutions :—0·4, 0·35, 0·3, 0·25, 0·2, 0·15, 0·1, 0·09, 0·08 to 0·01%. Two c.c. of each of these solutions are measured into uniform small test tubes with drawn-out ends ready for sealing. Three-tenths of a c.c. of 30% trichloracetic acid are added to each. They are then boiled, and the tubes are sealed. To make the test, 2 c.c. of fluid are pipetted out into a similar tube, 0·3 c.c. of 30% trichloracetic acid is added, and the mixture is boiled. After thorough shaking the opacity is matched with the standards, and the percentage can be arrived at. The protein content may also be estimated in a small-sized Esbach tube. If the protein content be increased above 0·5%, and if there be no pus, and, in addition, a yellowish coloration (xanthochromia), obstruction to the spinal subarachnoid space should be suspected, thus preventing the normal circulation of cerebro-spinal fluid. This is known as *Froin's syndrome* (see p. 337).

Quantitative Chemical Analysis of the Fluid

The reaction should first be determined by indicators. This is usually about *p*H 7·45. In some infections, the organisms are said to ferment the glucose present, thus producing acid and lowering the *p*H.

For the analysis, we recommend proceeding by Folin

and Wu's methods, as in the case of blood, except that the volume is made up as follows :—

1 volume of fluid.
$8\frac{1}{2}$ volumes of water.
$\frac{1}{4}$ volume of sodium tungstate.
$\frac{1}{4}$ volume of $\frac{2}{3}$ N sulphuric acid.

The mixture is filtered, and the usual estimations are performed. Of these urea, non-protein nitrogen, and sugar are of importance. Chlorides are estimated by the method given on p. 389 for blood chlorides.

Normal figures, given by various observers, differ very considerably, but the following table represents the general opinion :—

Alkali reserve 58 to 63 . .	McClendon (19)
*p*H fresh . 7·4 . . .	Tashiro & Levinson (20)
*p*H on standing 8·3 . . .	Leopold & Bernard (21).
Urea 15 to 30 mg. per 100 c.c. .	,, ,,
Creatinin 0·7 to 1·5 mg. per 100 c.c.	,, ,,
Dextrose 70 to 100 mg.	,, ,, ,, ,,
Chloride 700 mg.	,, ,, Mestrezat (22).

We have usually found the nitrogen figures to be higher than the above, and more approaching the values for blood.

The urea, creatinin and non-protein nitrogen figures are increased in nitrogen retention. According to our personal experience, the blood and cerebro-spinal fluid nitrogen contents do not always correspond, although when the blood figures rise the fluid figures also increase. Thus it is quite common to find a fluid containing much more urea and non-protein nitrogen than the blood. In uræmia the figures are high, usually over 100 mg. per 100 c.c., although this is not invariable. We have seen patients in uræmia with a non-protein nitrogen content in the fluid of only 68 mg. per 100 c.c., and others without uræmic symptoms, when the fluid contained 200 mg. per 100 c.c. In the vast majority of cases, however, the non-protein nitrogen figures are well over

100 mg. per 100 c.c., and up to 500 mg. per 100 c.c. It is very unwise to give any statement on the imminence of uræmia based upon analysis of the cerebro-spinal fluid.

Quantitative estimation of the sugar content is said to be of diagnostic importance, since it is absent, or greatly reduced, in all conditions of acute suppurative meningitis. This is said to be due to the fact that organisms ferment the sugar. Kopetsky (23), Schloss (24), and others, have reported absence or diminution of sugar in all forms of acute meningitis. The sugar content is also stated to be slightly diminished in syphilitic lesions of the central nervous system (Hopkins (25)). In diabetes mellitus the sugar content is increased in proportion to the blood sugar.

Chlorides are increased in nephritis (Levinson (26)), and decreased in meningitis. Tuberculous meningitis is usually accompanied by a particularly low chloride content, 500 mg. per 100 c.c. or less.

A summary of the properties and composition of various pathological fluids is given at the end of the chapter.

Cytological Examination

The fluid must not be more than a few hours old, otherwise the cell count will be valueless. Purves Stewart recommends that 5 c.c. should be centrifuged and the supernatant fluid carefully decanted. The deposit is then transferred to a slide by means of a capillary pipette and is stained by methyl blue or Jenner's stain.

The cells should be counted in the ordinary way, using undiluted fluid. For absolute accuracy the special technique and chamber of Fuchs and Rosenthal are recommended. Ten c.mm. of fluid are diluted with 1 c.mm. of staining fluid (methyl violet and acetic acid) in a pipette. After mixing, the cells are enumerated in the chamber. A summary of the findings will be found in the table at the end of this chapter.

Conclusions.—The value of the Wassermann reaction, cell

count, and globulin tests is beyond doubt. With regard to the colloidal gold reaction, opinions differ, and many authorities maintain that it can add little information of value to that afforded by Nonne's "four reactions." The technique is not without difficulty, and it would appear to be unwise to employ it except in conjunction with the "four reactions."

Ferguson Watson (27) states that the colloidal gold reaction is positive in only 85% of cases of nervous syphilis, whereas the Wassermann reaction is positive in 95%, and that paretic and luetic responses are given in non-syphilitic conditions such as encephalitis lethargica, disseminated sclerosis and influenza.

With regard to other colloidal reactions, such as the colloidal benzoin and the mastic reaction, Greenfield and Carmichael (28) state that they are not so sensitive as is the colloidal gold reaction.

Nonne employed four reactions in the investigations of syphilitic lesions of the central nervous system.

These are :—

Wassermann reaction on cerebro-spinal fluid.
Wassermann reaction on blood.
Cell count.
Nonne-Apelt reaction, phase 1.

The following table, compiled from various sources and modified slightly, expresses the results obtained in various syphilitic conditions :—

Condition.	*Wassermann reaction in C.S.F.*	*Wassermann reaction in Blood.*	*Pleocytosis.*	*Nonne-Apelt reaction, Phase 1.*
General paralysis . .	100% +	100% +	95% +	95–100% +
Cerebro-spinal syphilis .	65% +	80–90% +	100% +	Negative.
Tabes dorsalis without paralysis . . .	55% +	60–70% +	100% +	85–90% +

It must be understood that these figures are only approximate, and are intended to give a general impression rather than an exact statistical statement. Fildes and McIntosh (29), after an extensive investigation into the application of the Wassermann reaction to neurological problems, came to the following conclusions :—

1. In dementia paralytica and tabo-paresis a strongly positive reaction is almost always found in both the blood serum and the cerebro-spinal fluid. A negative reaction would render the diagnosis doubtful.

2. In tabes dorsalis very similar results are obtained, but the reaction in the cerebro-spinal fluid is usually weaker than in paresis.

3. In cerebro-spinal syphilis the results vary according to the site of the lesion. If the cord be involved the Wassermann reaction on the fluid is usually very strongly positive, but if the cord be not affected, the reaction may be slight or absent.

4. Treatment affects the findings according to its efficacy.

5. In syphilitic conditions not associated with nervous lesions the reaction is negative.

It would appear that the Wassermann reaction becomes positive in the cerebro-spinal fluid owing to leakage of serum from the blood. This contention is supported by the observation that if a person with syphilis have an attack of meningitis, the Wassermann reaction, on the cerebro-spinal fluid, which was previously negative, will become positive.

The value of the colloidal gold reaction is upheld by most observers, but this reaction yields the best results in syphilitic lesions. Some consider it to be more delicate than the Wassermann reaction.

Chemical examination is useful in that it provides confirmatory evidence, but its value is doubtful if taken alone.

On p. 352 will be found a table showing the findings in a series of conditions. It is compiled from Nonne's, Plaut's and Levinson's works.

Table Showing Composition of Cerebro-spinal Fluid in Various Conditions

Type of case.	Amount easily removed and pressure in mm. H_2O.	Cells per c.mm. Type.	Nonne-Apelt.	Total Protein. %	W. R.	Colloidal gold.	Chemical.				
							Reaction. *p*H.	Urea. mg. per 100 c.c.	N. P. N. mg. per 100 c.c.	Chlorides. mg. per 100 c.c.	Sugar. mg. per 100 c.c.
Normal.	7–10 c.c. { 90 mm. (child). 150 mm. (adult).	1–5 mono-nuclears.	—	0·015 to 0·03	—	—	7·45	20	25	720–750	100
Meningococcal meningitis.	30–50 c.c. 300–700 mm.	50 to 3,000 polymorphs.	+	0·05 to 0·5	—	+ Meningitic curve.	6	30	35	650–700	0–30
Acute anterior poliomyelitis.	10–50 c.c. 300 mm.	10 to 100 polymorphs, later mono-nuclears.	+	0·05 to 0·2	—	—	7·45	20	25	720–750	100
Tuberculous meningitis.	15–30 c.c. 300–700 mm.	30 to 400 mono-nuclears.	+	0·05 to 0·25	—	+ Meningitic curve.	7	35	40	500	6–40
Loculation syndrome.	Varies.	Varies with cause.	+	0·1 to 0·4	Varies. with cause.	Varies with cause.	7·45	50	45	680	70–100
General paralysis.	7–20 c.c. 160 mm.	10 to 50 mono-nuclears.	+	0·05 to 0·1	+	+ Paretic curve.	7·45	20	25	720–750	100
Cerebro-spinal syphilis.	7–20 c.c. 160 mm.	10 to 50 mono-nuclears.	+	0·05 to 0·08	+	+ Luetic curve.	7·45	20	25	720–750	100

REFERENCES

1. Ayer.
Arch. Neurol. and Psych., 1920, iv., 529.
2. Dixon and Halliburton.
Journ. Physiol., 1913, xlvii., 215.
3. Wegeforth, Ayer and Essick.
Amer. Journ. Med. Sci., 1919, clvii., 789.
4. Ayer.
Trans. Amer. Neurol. Assocn., 1921, 272.
5. Queckenstedt.
Ztschr. f. Nervenheilk., 1916, lv., 325.
6. Froin.
Gaz. d. Hôp. de Paris, 1903, lxxvi., 1005.
7. Sicard and Forestier.
Rev. Neurol., 1921, xxviii., 1264.
8. de Martel.
Rev. Neurol., 1923, xxx^{ii}., 444.
9. Sargent.
Brit. Med. Journ., 1923, ii., 174.
10. Ayer and Mixter.
Arch. Neurol. and Psych., 1924, ii., 499.
11. Armour.
Lancet, 1927, i., 423.
12. Sharpe and Peterson.
Ann. of Surg., 1926, lxxxiii., 32.
13. Sicard and Forestier.
Presse Medicale, 1923, xxi., 493.
14. Nonne.
"*Syphilis und Nervensystem*," 1909, 607–641. Karger, Berlin.
15. Lange.
Zeit. f. Chemotherap., 1912, i., 44.
16. Cruickshank.
Brit. Journ. Exp. Path., 1920, i., 71.
17. Black, Rosenberg and McBride.
Journ. Amer. Med. Assocn., 1917, lxix., 1855.
18. Nonne-Apelt.
Arch. f. Psych. Bd., 43, H. 2.
19. McClendon.
Journ. Amer. Med. Assocn., 1918, lxx., 977.
20. Tashiro and Levinson.
Journ. Infect. Dis., 1917, xxi., 571.
21. Leopold and Bernard.
Amer. Journ. Dis. Child., 1917, xiii., 34.
22. Mestrezat.
Bull. Chem. Soc., 1911, ix., 683–688.
Journ. de Physiol., 1913, xiv., 504.

23. Kopetsky.
"*Manhattan Eye, Ear and Throat Hospital Rep.*," 1913, 397.
24. Schloss.
Quoted in "*Cerebro-spinal Fluid*" (Levinson), Chapter V., 1919. Kimpton, St. Louis.
25. Hopkins.
Quoted in "*Cerebro-spinal Fluid*" (Levinson), Chapter V., 1919. Kimpton, St. Louis.
26. Levinson.
"*Cerebro-spinal Fluid*," 1919. Kimpton, St. Louis.
27. Ferguson Watson.
Quart. Journ. Med., 1926, xix., 431.
28. Greenfield and Carmichael.
"*The Cerebro-Spinal Fluid in Clinical Diagosis*," 1925. Macmillan & Co., Ltd., London.
29. Fildes and McIntosh.
"*The Wassermann Reaction and its Application to Neurology.*" *Brain*, 1913, xxxvi., 193.

REFERENCE BOOKS

Plaut.
"*Leitfaden zur Untersuchung der Zerebrospinal flussigkeit*," 1920. Fisher, Jena.
Nonne.
"*Syphilis und Nervensystem*," 1909. Karger, Berlin.
Levinson.
"*Cerebro-spinal Fluid*," 1919. Kimpton, St. Louis.
Mestrezat.
"*Le liquid cephalo-rachidien normal et pathologique*," 1912. Masson, Paris.

CHAPTER XIV

SPECIAL BLOOD EXAMINATIONS; THE LIVER TREATMENT OF PERNICIOUS ANÆMIA

Estimation of the Fragility of the Red Blood Corpuscles

Solutions.—An accurate 1% solution of NaCl. This is made by dissolving 1 g. in about 90 c.c. of distilled water and diluting to the required strength after titration with silver nitrate and potassium thiocyanate solutions.

Method.—A double row of tubes is set up in a rack and is filled according to the scheme shown.

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1% saline, c.c. .	0·6	0·7	0·8	0·9	1·0	1·1	1·2	1·3	1·4	1·5
Water, c.c. . .	1·4	1·3	1·2	1·1	1·0	0·9	0·8	0·7	0·6	0·5
The resulting saline content being expressed in percentages.	0·3	0·35	0·4	0·45	0·5	0·55	0·6	0·65	0·7	0·75

0·1 c.c. of the patient's blood is added to each tube in the front row, whilst a similar quantity of normal blood is added to each of the back row tubes to form a control. The test is recorded by + signs, + + + representing complete hæmolysis, + + partial, and + commencing hæmolysis.

Findings.—Normal blood is found to hæmolyse constantly at 0·45% saline.

At this concentration there are always intact corpuscles at the bottom of the tube, *i.e.*, hæmolysis is incomplete.

There are still a few corpuscles left in the 0·4 tube, but at 0·35% hæmolysis is usually complete.

Acholuric jaundice is the only disease in which the cor-

puscles are constantly found to be pathologically fragile. This is true in both the congenital and acquired forms. It is usual to find that there is slight hæmolysis at 0·7% and 0·65%. The process is usually complete at 0·5%. Observers have recorded slight increase in fragility in some forms of pyæmia, such as the streptococcal variety, and also in von Jaksch's *anæmia pseudo-leukæmia infantum*. In all the recorded cases hæmolysis occurred at 0·5% but not in stronger solutions. The picture is therefore quite different from that obtained in acholuric jaundice.

In some cases an increased resistance to hæmolysis may be found in the red blood corpuscles. This type is usually met with in cases of obstructive jaundice, and more especially in those cases where the obstruction is due to malignant growths of the pylorus, liver or pancreas. It is encountered in all cases of splenic anæmia and also after splenectomy.

Since there is never any increase in fragility of the red cells in splenic anæmia, the test performs a useful function in the differential diagnosis of acholuric jaundice and splenic anæmia. In all other diseases, both general and of the blood, there is no alteration of the corpuscular fragility.

Very interesting results have been recorded on the effects of operation on cases of acholuric jaundice. Thus, after splenectomy the increase in fragility has been found to disappear, and in many cases to be replaced by an increase in resistance to hæmolysis. Even after many years there is no return of fragility.

With regard to the cause of this undue fragility there is considerable difference of opinion, but the majority of observers are convinced that it lies in the corpuscles themselves and not in the serum.

An account of the clinical applications of this test will be found in a paper by Thursfield (1), and a more general description appears in Wells' " Chemical Pathology " (5th edn.), p. 256.

Blood Grouping

With the increasing recognition of the importance of blood transfusions, the determination of the compatibility of two bloods becomes of the greatest importance. It was known many years ago that the intravenous injection of blood was attended with fatal results in a certain number of cases. Research into this point led Jansky (2) and Moss (3) to separate individuals into four groups, according to the interaction of their sera on corpuscles. The interactions may be expressed by the following scheme :—

Group I. serum agglutinates no red cells.
„ II. „ „ red cells from Groups I. and III.
„ III. „ „ red cells from Groups I. and II.
„ IV. „ „ red cells from Groups I., II. and III.

Or, conversely,

Group I. cells are agglutinated by sera of II., III. and IV.
„ II. „ „ „ III. and IV.
„ III. „ „ „ II. and IV.
„ IV. „ are not agglutinated by any sera.

The distribution of the population in these groups is variously stated, but the majority of workers agree to something like the following :—

Group I.	7%
„ II.	40%
„ III.	10%
„ IV.	43%

There is some difficulty with regard to the classification, since Jansky and Moss worked independently. Moss's classification is the one usually adopted, but some observers adhere to Jansky's classification, which transposes Groups I. and IV., Groups II. and III. remaining the same in both.

Method of Grouping.—Stock specimens of sera II. and III. are necessary.

A perfectly clean slide is taken, and a drop of serum II., and one of serum III., are placed on it at either end. These two drops are diluted with one drop of saline, and a drop of the blood to be tested is added to each and stirred with a platinum loop. If agglutination take place, the corpuscles can be seen to clump by the naked eye, or by a low-powered microscope, and the smear becomes flaky. From the result the group can be predicted.

If agglutination occur with serum III. alone the blood is Group II., if with II. alone it is a Group III.

If no agglutination occur at all, the unknown blood belongs to Group IV., and if with both to Group I. This is according to Moss's classification.

The ideal condition is to transfuse patients of the same group, but if this be not possible, IV., being a universal donor, will answer the purpose well.

In addition to the testing with sera II. and III., it is always advisable to perform a direct test with the patient's own blood and that of the donor. Two drops, one from each blood, are mixed on a slide, and after the lapse of some minutes the preparation is inspected for agglutination. Preferably, the donor's cells should be tested against the recipient's serum, and *vice versâ*. Even if the groups of the donor and recipient be compatible according to tests with sera II. and III., untoward results have occurred, and agglutination has been found by the direct test. This is claimed by some to be due to the existence of subgroups, but the ill effects can be avoided if a preliminary direct test be performed.

The necessity for absolutely fresh standard sera cannot be too strongly emphasised, since they deteriorate rapidly if kept at room temperature. Many fatalities have occurred through incorrectly grouping a donor as a " four " by means of inactive sera II. and III. These should be as fresh as

possible, and should be stored in a freezer, frozen solid. An ordinary ice chest is insufficient. It is always advisable to perform a preliminary control with a known serum, preferably a I., II., or III. group, since by this means the activity of the standards can be guaranteed.

If a patient be transfused with blood from an incompatible group, he experiences distress and pain in the back if conscious, and if anæsthetised, there is usually respiratory distress and weakening of the pulse. If a large quantity of blood be transfused, then the patient becomes extremely collapsed, and death usually occurs in a few days, with uræmic symptoms, and hæmoglobinuria. The mechanism is that the donor's red corpuscles become rapidly agglutinated and hæmolysed. The resulting hæmoglobin is excreted by the kidneys, and becomes precipitated in the renal tubules, with the production of an intra-renal obstruction, and death from uræmia. S. L. Baker and one of us (E. C. D.) were able to study some fatal cases of this type. Observations were made on the blood urea, which in one instance rose to 900 mg. per 100 c.c. at death. The kidneys, on section, showed deposits of brown pigments in the tubules with dilatation. Since the mechanism of the precipitation of hæmoglobin in the tubules had not been satisfactorily determined, an attempt was made to arrive at the exact cause. A very detailed study was made of the whole problem, but there is only sufficient space here to summarise the paper (4). There are two conditions necessary for the precipitation of hæmoglobin in these circumstances, an acid urine about *p*H6, and a concentration of salts up to 1%.

If these conditions prevail, then an insoluble brown precipitate appears, exactly similar to that composing the casts in patients with hæmoglobinuria. We therefore concluded that the hæmoglobin produced by lysis of the donor's cells is excreted by the glomeruli and remains in solution in the dilute, slightly alkaline glomerular transudate. When the fluid reaches the tubules re-absorption begins to take place,

and the acidity and salt content rise, with resulting precipitation.

All these conclusions were arrived at from a careful study of animal experiments, and since bringing about dilution of the urine and decreasing its acidity caused recovery in our obstructed animals through dissolving the precipitate, it is suggested that massive alkaline transfusions in human cases might prevent the formation of intra-renal obstruction. The actual compound formed in the tubules is a variety of acid hæmatin ; details of the chemical side of the investigations will be found in the paper (4). Witts (5) suggests that alkalies should be given by mouth in sufficient doses to render the urine alkaline before every blood transfusion.

It can be seen, therefore, that very serious results may occur through a mistake in grouping, and that the utmost attention must be paid to the remarks on the preceding pages. For a full account of the subject, see references (6) and (7).

Sedimentation of Red Cells

Fahraëus (8) was one of the first to make observations upon the rate of sedimentation of erythrocytes in citrated blood. He showed that in pregnancy the red cells sink much more rapidly than they do normally, and he called that property, which is shown by differences in the sinking speed of the red cell, the "*suspension-stability*" of blood. A slow rate of sinking indicates a high degree of stability. He used the method of vein puncture, running the blood directly from the needle into a small tube containing 2 c.c. of 2% sodium citrate solution. The tube is 17 cm. long, with an internal diameter of 9 mm.,and there is a mark indicating a content of 10 c.c. situated about 2 cm. from the top of the tube. The blood is run in to this 10 c.c. mark. The contents are mixed by inversion, the tube then placed vertically, and the rate of sedimentation of the red cells estimated by the depth of the clear fluid which appears on the top at the end

of one hour. Fahraëus found that the sedimentation rate was increased in " all kinds of infection, most distinct when accompanied by high fever, in many cases of malignant tumours and certain species of psychoses, etc." In Linzemeier's (9) method the tubes are 3 to 4 mm. in diameter and 6·5 cm. high, with a mark indicating the level of 1 c.c. of fluid.

This mark is indicated by the figure 0, and below this marks are made at 6, 12 and 18 mm. respectively. Five per cent. sodium citrate is used, 0·2 c.c. being drawn up into a syringe, the vein is then entered and blood drawn up to the 1 c.c. mark. The citrated blood is mixed by drawing in a bubble of air and inverting the syringe several times. The citrated blood is then placed in the tube and reaches up to the 0 mark. The red cells fall to the bottom and the time is noticed for their upper level to fall to the 6, 12 and 18 mm. mark. In a normal individual it takes between 250 and 300 minutes for the red cells to sediment to the 18 mm. line.

Westergren (10) modified the Fahraëus technique and carried out an important series of observations on the sedimentation rate in tuberculosis. He used a record syringe holding 1·2 c.c., furnished with a small catch on the piston and having a fine needle with an external diameter of 0·7 mm. The syringe is filled without the needle, with 3·8% sodium citrate solution, the needle is then attached and the piston pushed down until its clasp catches, when the syringe is one-fifth full. The vein is then entered and the syringe filled with blood. The citrated blood is passed through the needle into a small 2 c.c. test tube and mixed by inversion. The actual sedimentation test is performed in a special glass pipette. This consists of a dry and clean glass tube, 30 cm. long, and having an internal diameter of 2·5 mm., the lower end being drawn out into a short narrow point. A mark is placed on it 200 mm. from its lower end, and the citrated blood is sucked up to the mark, the contents then being about 1 c.c. The blood should be sucked up to the mark

and out again at least five times to ensure proper mixing. The pipette, thus filled to the mark, is placed in a special rack, standing vertically with the lower end on a piece of rubber and its upper end held by a steel spring. The time is noted. Readings are taken at the end of one and two hours, the column of clear fluid above the sedimented corpuscles being measured. The measurement is taken from the lower edge of the meniscus of the free surface of the liquid to the upper border of the corpuscles. If the upper border be not sharply defined the reading is taken down to a point a little lower, where the density is obviously complete. In order to obtain the average hourly sedimentation rate for the first two hours, the formula of Katz (11) may be employed. It is :—

$$\frac{S_1 + \frac{S_2}{2}}{2.}$$

where S_1 = the column of clear fluid in mm. at the end of one hour, and S_2 its height at the end of two hours.

Westergren carried out observations on 340 male patients suffering from tuberculosis in a sanatorium at Stockholm.

He gives the following figures for the classes into which observations fall :—

1. Clear fluid column of 3 mm. is normal.
2. Clear fluid column of 4 to 6 mm. is doubtful.
3. Clear fluid column of 7 to 12 mm. is probably pathological.
4. Clear fluid column of over 12 mm. is certainly pathological.

Zeckwer and Goodell (12) have more recently described a method which they have employed in America, which does not involve the use of such elaborate apparatus. The sedimentation tube consists of an ordinary 15 c.c. glass centrifuge tube graduated in tenths of a c.c. Two c.c. of 3% sodium citrate solution are first placed in the tube, a vein

is then entered with a fine needle and blood allowed to run into the centrifuge tube to the 10 c.c. mark. The tube is then inverted and stood vertically. A reading is taken at the end of one hour. In this method the height of the column of sedimented red cells is read and not the supernatant clear fluid, as in the other methods. Thus if the sedimentation be very marked the red cells will occupy about 3 or 4 c.c., whereas if the sedimentation be normal the red cells will extend up to the 7 or 8 c.c. line.

Observations were carried out upon 125 cases, and they were divided into four groups, according to the degree of sedimentation.

1. Red cells occupy 2 to 4·5 c.c.
2. ,, ,, 4·5 ,, 5·5 ,,
3. ,, ,, 5·5 ,, 8·0 ,,
4. ,, ,, 8 ,, 9·8 ,,

The authors claim that as only comparatively gross alterations in degrees of sedimentation are of significance, there is no necessity to employ sedimentation tubes of fine calibre, as differences of a few millimetres can be neglected.

They obtained the following results, all with one hour observations :—

	Average reading.
Normal	Red cells occupy 7·1 c.c.
Pregnancy . . .	,, ,, 5·8 ,,
Tuberculosis . . .	,, ,, 4·4 ,,
Acute inflammatory processes, other than tuberculosis	,, ,, 4·3 ,,
Malignant tumours . .	,, ,, 4·2 ,,

We have employed the method of Zeckwer and Goodell in estimating the rate of sedimentation of the erythrocytes in various conditions, and our results correspond fairly closely with those detailed above.

The Nature of the Reaction.—Fahraëus (8) showed that

the sedimentation is due to the action of hæmagglutinins, the red cells when agglutinated dropping down. Hæmagglutination has been found to depend upon the presence of proteins in the plasma. Thus sedimentation occurs very rapidly in pure fibrinogen, less rapidly with globulin and very slightly in solutions of albumen. A reduction in the suspension-stability of blood is brought about by an increase in the fibrinogen or globulin protein fractions. Breaking down of tissue proteins may therefore account for an increased rate of sedimentation.

The Value of the Test.—It must be clearly understood that the stability reaction or sedimentation test is not a special test for any disease. It is not therefore in any sense diagnostic, for, as has been shown above in diseases such as tuberculosis, carcinoma, acute inflammation and toxæmias, the rate of sedimentation is accelerated. However, it is possible that the test may have some value in such a disease as tuberculosis. Thus Westergren (10) states that a normal sedimentation is never given in active tuberculosis. If the converse be shown to be true the test will obviously be of great value in the investigation of that large number of cases in which the patient is run down and doubtful signs are found at one apex of the lungs, the sputum contains no tubercle bacilli, the temperature is within normal limits, the X-rays show increased root shadows with diminished translucency of one apex, and the complement fixation test is either positive or negative. Westergren suggests that with a normal stability reaction an active phthisis can probably be excluded, and further observations on this point are required. In one of our cases, however, in which tubercle bacilli were present in the sputum, and the temperature varied from 97° F. (morning) to 99° F. (evening) while the patient was in bed, the sedimentation figure was 9·4 c.c. red cells at one hour, using the Zeckwer and Goodell method. Activity was undoubtedly present in this case and yet the sedimentation test was negative.

Apart from this it is doubtful whether the stability reaction will be found to yield more information in tuberculosis than is already afforded by the clinical picture of the case. It is said that the reaction is a rather more delicate indication of activity than is the temperature chart, and that the stability is diminished before the temperature rises. During convalescence and sanatorium treatment the stability reaction may also lag behind the other signs of diminished toxæmia, so that the patient may apparently have arrested disease as judged by the physical signs and symptoms, and pulse and temperature exercise responses, and yet the sedimentation test may still show diminished blood stability. In such instances it is claimed that arrest cannot be considered to be firmly established until the sedimentation rate has returned to normal.

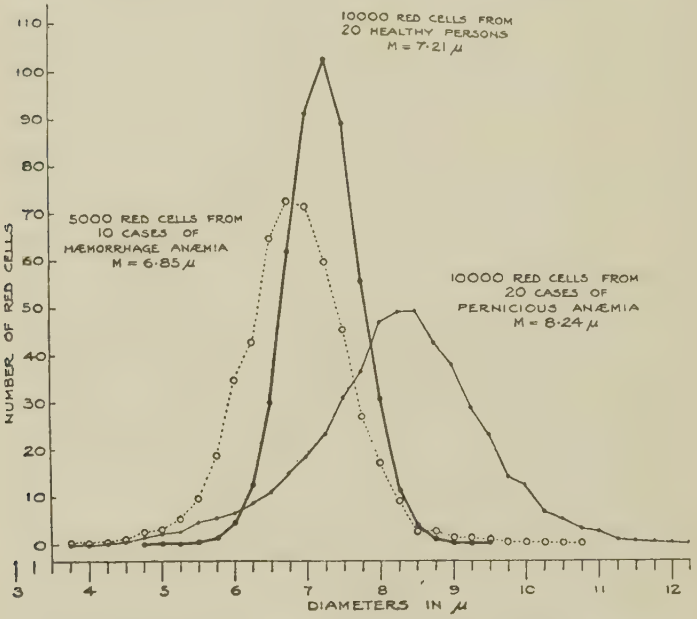
In diseases such as cancer, an actively growing and fungating tumour gives a greater sedimentation rate than does a slowly growing scirrhous carcinoma. This is probably dependent upon the amount of tissue destruction and subsequent increase in fibrinogen and globulin in the blood.

The sedimentation test has therefore many limitations, but it appears to have some value as an aid to diagnosis and prognosis if used intelligently in conjunction with other clinical and laboratory investigations.

Estimation of the Size of the Red Cells in Anæmias

It has long been known that inequality in the size of the red cells in pernicious anæmia is a characteristic feature of the disease. Price-Jones (13) has elaborated a method for demonstrating this graphically. A series of cells, usually 500, are measured and the results are plotted upon a curve, the ordinates being graduated in terms of mean diameter and number of cells measured respectively. A thin film is made and dried in air. It is then stained with Jenner's fluid

for two minutes, followed by eosin for a similar period. This intensifies the original stain. The film is then placed upon the stage of a microscope fitted with some suitable form of projection apparatus, such as a camera obscura or projection prism. The magnifications are so arranged that the cells, as



Price-Jones.

FIG. 44.—The curves of pernicious anæmia and of anæmia following hæmorrhage stand on a wider basis than does the normal one, but the former is shifted to the right and the latter to the left.

appearing on the paper after projection, are magnified 1,000 diameters. A measurement of 1 mm. on the paper is thus equal to 1μ on the slide. The maximum and minimum diameter of each cell is measured with a millimetre scale, and the mean is taken and converted into μ measurements. The cells are then classified in groups progressing by $0{\cdot}25\ \mu$ in diameter, and the mean diameter of 500 cells is taken to

represent the mean diameter of the red cells of any specimen of blood.

Normally the cells vary from 6 to 8·75 μ in diameter, with a mean of 7·2 μ for 10,000 cells from twenty individuals. In pernicious anæmia the red cells vary from 4 to 12 μ, and the mean diameter of 10,000 cells from twenty cases is 8·24 μ. In the secondary anæmia following hæmorrhage, on the other hand, the mean diameter of the red cells is smaller than in health, although some macrocytes are present, the measurement being 6·85 μ in a series of 5,000 cells from ten cases.

The anisocytosis in pernicious anæmia is therefore in two directions, but as the macrocytes outnumber the microcytes the net result is an increase in the average size of the red cells. This megalocytosis is a characteristic early feature of the disease, and also persists during the remissions, although the total number of the red cells may be nearly normal. Similar curves to those obtained in pernicious anæmia have been observed by Shackle and Hampson (14) in the anæmia of sprue, and by Passey and Carter Braine (15) in anæmia secondary to infection with the dibothriocephalus latus.

Variations of 0·5 μ occur in healthy people during the day, the cells being smaller in the morning than in the evening. Violent exercise increases the diameter, which corresponds with an increase in volume. The size of the cells is found to vary with the reaction of the blood, an increased acidity causing an increase in their size and *vice versâ*.

Reference to the appended curves will render the value of these observations clear (see Fig. 44).

The Liver Treatment of Pernicious Anæmia

The use of liver in the treatment of pernicious anæmia constitutes a therapeutic advance of similar importance to the discovery of insulin for the treatment of diabetes. There is little doubt that the origin of the idea can be found in the researches of Whipple (16) and his collabora-

tors. These workers produced a severe secondary anæmia in dogs by bleeding, and proceeded to study the effects of various food substances on blood regeneration. They found that if liver were added in large quantities to the diet, there was a remarkable increase in the blood count which appeared to be entirely due to the liver. Shortly after the publication of these results, Minot and Murphy (17) described the application of the same principles to the treatment of pernicious anæmia. In 1926 they reported the effect of a diet rich in liver on 45 cases of pernicious anæmia. These results were very striking indeed, since they showed that one month's treatment increased the blood count by some 2,000,000 of red cells. A series of publications followed, with the result that the regimen was fully upheld, and now several years' experience has proved that the blood picture can be restored almost to normal in practically all patients, and further, provided that the liver diet is continued, the patients remain free from symptoms.

The liver is best administered in the raw condition, at the rate of 240 g. or ½ lb. daily. Ox liver is most efficacious, but good results are obtained with calf or pig liver, which must in all cases be fresh. It may be given in either two or three doses during the day. Liver is most potent when administered as a pulp, and can be taken in this way raw, in a cupful of water, flavoured with orange juice. It may also be given raw as a sandwich flavoured with a sauce. Raw liver, cut up, may be added to scrambled eggs or to soups. Good results are also gained with cooked liver provided a high temperature is avoided and that the time of cooking is as short as possible. Various recipes for liver served as sandwiches, or fried, curried, minced or added to purées and soups are given by Pollock (18).

As an example may be quoted the following ingenious recipe useful for travelling purposes :—

"*Liver Roll.*—Mince together 12 oz. of liver, 6 oz. of beef (preferably steak), and 6 oz. of rather lean bacon. Add a

trace of finely chopped onion. Mix a teacupful of bread-crumbs with the minced liver and bacon. Beat up one egg and stir it into the liver mixture, flavouring the whole with a little tomato sauce. Wrap it in greaseproof paper, and cook in a steamer from one to one and a half hours. Let it get cold and eat it as a sandwich or in place of cold meat."

Minot says that the liver may be cooked in any way provided that prolonged boiling is avoided. There appears to be no necessity to give hydrochloric acid by mouth, and the strict reduction of fats, and to a lesser degree of carbohydrates, as originally proposed by Minot and Murphy, is not now considered essential.

During the last few years a series of fluid extracts and solid water-soluble concentrates made from liver has appeared on the market. Cohn (19) succeeded in preparing a concentrated extract of liver by a process of acid digestion and alcohol precipitation, and this will protect the patient when given in doses of 5 to 10 g. per day. It is free from the higher proteins, and may thus be used in cases of pernicious anæmia associated with nephritis and nitrogen retention, in which a high liver diet would be inadvisable. There are on the market several preparations of this nature approved by the Medical Research Council. The use of these products obviates the difficulties of intolerance and nausea, since the powder may be added to soup or sprinkled over food, but the chief objection to them lies in the expense. It is usually possible to alternate the administration of $\frac{1}{2}$ lb. of crude liver with the extract when the patient feels unable to take the former. The extracts are also particularly useful when owing to soreness of the tongue the patient cannot masticate without pain.

Very little can be stated with regard to the mode of action. It has been suggested by Peabody (20) that the liver secretes a hormone that is necessary for the maturation of the red cells in the bone marrow, and that the lack of this substance

causes the appearance of immature red cells in the circulation. According to this theory the liver acts directly on the bone marrow. The changes produced in the blood as the result of successful treatment include the restoration of a normal red cell count, return of the colour index to normal, an increase in the number of white cells with disappearance of the relative lymphocytosis. Special attention is directed to the reticulocytes present in the blood. These are immature red cells, normally present in blood to the extent of 0·1 to 1% of the red cells. They cannot be seen in dried stained films, but are demonstrated by "vital" staining in wet films. A blood film is made on a slide, which has previously been coated with a thin dried film of a dye, such as cresyl-blue. A cover slip is then placed on the blood film, which is examined wet (Davidson *et al* (21)). The reticulocytes contain a network, granules or dots, stained blue. They probably correspond with the red cells showing polychromasia in the ordinary dry films. In untreated pernicious anæmia the reticulocytes are usually somewhat increased in numbers, as 2% or more of the red cells. In cases of pernicious anæmia treated with liver, which are going to respond well, the reticulocytes rise rapidly to 15 or 30% in a week, and then fall suddenly (reticulocyte crisis) to reach normal in about three weeks. The increase in the red cell count begins usually about the time of the reticulocyte crisis, and persists for several weeks after. The excess of bilirubin in the serum disappears early, at the time of the rise in the reticulocyte count, as shown by Dyke and Greener (22). An increase in the eosinophils has been noted by Whitby (23) and other workers; this usually reaches a maximum, which may be as high as 26%, between the eighth and twelfth weeks of treatment.

Achlorhydria persists after liver treatment, and the response of the nervous symptoms is variable. Early ataxic lesions may improve, and numbness disappear, but the later spastic conditions are, generally speaking, unaffected.

REFERENCES

1. Thursfield.
St. Bart.'s Hosp. Journ., 1920, xxviii., 37
2. Jansky.
S'born. Klin., 1907, viii., 85, quoted in *Journ. Amer. Med. Assocn.*, 1921, lxxviii., 130.
3. Moss.
Johns Hopkins Hosp. Bull., 1910, xxi., 63.
4. Baker and Dodds.
Brit. Journ. Exp. Path., 1925, vi., 247.
5. Witts.
Lancet, 1929, i., 1297.
6. Keynes.
" Blood Transfusion," 1922. Oxford Univ. Press, London.
7. Guthrie and Huck.
Johns Hopkins Hosp. Bull., 1923, xxxiv., 128.
Guthrie and Pessel.
Ibid., 1924, xxxv., 81.
8. Fahraëus.
Biochem. Zeit., 1918, lxxxix., 355. *Acta med. Scand.*, 1921, lv., 1.
9. Linzemeier.
Pfluger's Arch. f. d. ges. Physiol., 1920, clxxxi., 169.
10. Westergren.
Acta med. Scand., 1921, liv., 247.
Brit. Journ. of Tuberc., 1921, xv., 72.
11. Katz.
Zeit. f. Tuberk., 1922, xxxv., 401.
12. Zeckwer and Goodell.
Amer. Journ. Med. Sci., 1925, clxix., 209.
13. Price-Jones.
Brit. Med. Journ., 1910, ii., 1418.
" *Blood Pictures*," 1920, 2nd ed. John Wright and Sons, Bristol.
Journ. Path. and Bacteriol., 1922, xxv., 487.
14. Shackle and Hampson.
Guy's Hosp. Rep., 1924, lxxiv., 193.
15. Passey and Carter Braine.
Guy's Hosp. Rep., 1924, lxxiv., 329.
16. Whipple et al.
Amer. Journ. Physiol., 1925, lxxii., 408.
17. Minot and Murphy.
Journ. Amer. Med. Assocn., 1926, lxxxvii., 470.
Ibid., 1927, lxxxix., 759.
Brit. Med. Journ., 1927, ii., 674.
18. Pollock.
Lancet, 1928, ii., 598.

19. Cohn et al.
Journ. Biol. Chem., 1927, lxxiv., 69.
20. Peabody.
Amer. Journ. Pathol., 1927, iii., 179.
21. Davidson et al.
Lancet, 1928, i., 847.
Ibid., 1928, ii., 1014.
22. Dyke and Greener.
Lancet, 1928, i., 1068.
23. Whitby.
Lancet, 1928, i., 285.

CHAPTER XV

BLOOD AND URINE ANALYSIS

Before commencing a detailed account of this important branch of biochemistry, it will perhaps be permissible to say a few words about the history of the subject.

One of the very first series of clinical blood analyses was performed by an Englishman in the year 1848. Sir A. B. Garrod (1) then published an account of the uric acid content of blood in cases of gout, and on his observations founded his now classical views with regard to the ætiology of that disease. Shortly afterwards, in 1850, Carl Schmidt (2) described his researches on the ultimate analysis of blood from fatal cases of cholera. Very little work of outstanding clinical value was done until after 1913, when Bang (3) introduced his micro-chemical method for the estimation of sugar. Folin (4), in 1913, began to publish his methods, out of which grew his present well-known system, described at length later. It is thus seen that blood analysis is of quite recent date, and it is a very significant fact that it has grown to its present importance in such a short time.

Before describing the various techniques, we have first to consider the collection of blood, for which there are two distinct methods: either by vein puncture or by capillary puncture. If anything like a complete analysis is to be attempted, vein puncture is the only method suitable. Every one is familiar with the details of this operation, but there are perhaps one or two points that might be emphasised.

Firstly, with regard to sterilisation of the syringe and

needle; any method other than by alcohol or ether is suitable. If there be no alternative to alcohol or ether, the syringe should be rinsed out before use with sterile water, otherwise the blood will clot. It is a good practice to keep a series of sterile syringes and needles in sterile cases. For general work we prefer to sterilise our needles in oil. Another important point, which is very frequently overlooked, is the necessity of having a sharp needle. There is, perhaps, no greater enemy to the blood chemist than a clinician armed with a blunt needle, since the operation becomes so painful that few patients will submit to it. A needle is not satisfactory unless it goes into the skin with the same readiness that it would into butter. The majority of new needles are quite blunt, and it is necessary to sharpen them on a piece of curved carborundum. This can easily be done with practice, and blood taking will lose its terrors, both for the patient and the operator. The blood should be transferred from the syringe to a small bottle or tube, containing a pinch of finely ground potassium oxalate. If too much oxalate be added, it will be found impossible to obtain complete coagulation of the proteins. About 20 mg. of potassium oxalate per 10 c.c. of blood are required to prevent clotting. If preferred, this may be added to the tubes prior to use in the form of a solution of potassium oxalate. Two drops of a 20% solution contain rather more than 20 mg. of oxalate. If the blood tube be treated, therefore, with 1 drop of such a solution, by "running" it over the sides of the tube, and allowing it to dry, the oxalate will be distributed in a finely divided form, and 10 c.c. of blood may be introduced without fear of clotting. Folin has recommended the use of small pieces of muslin (50 mg. of cloth per 10 c.c. of blood) which have previously been soaked in a solution made of lithium oxalate. This is prepared by dissolving 10 g. of lithium carbonate and 85 g. of oxalic acid in 1 litre of water, and evaporating to dryness. The residue, lithium oxalate, is dissolved in 240 c.c. of water, and the muslin strips are drawn

slowly through this solution. The question of glycolysis in normal blood must never be overlooked. In a careful research into this problem Tolstoi (5) has shown that sugar disappears very rapidly from normal blood but remains much more constant in diabetic blood at room temperature. His observations indicate that the disappearance of sugar in three hours is quite appreciable, and therefore estimations of blood sugar must be performed at once if accurate results are to be obtained. This is particularly important when a sugar tolerance curve is being performed, as if the specimens are all left until the end of the test period before the estimation of sugar is undertaken the shape of the curve will be materially altered by glycolysis in the earlier specimens. If sugar is to be estimated in the blood, it is advisable to add 1 drop of 40% formalin per 10 c.c. of blood if the determination cannot be made at once. Folin has shown that by this means glycolysis is prevented, and the sugar content remains constant for quite forty-eight hours. Although formalin is a powerful reducing agent, its addition to blood does not affect the alkaline copper solution. This may be due to its combination with the proteins. The tube is then corked and shaken.

Ten c.c. are sufficient for a complete analysis by Folin's method. If calcium is to be estimated, sodium citrate must be used in the same proportion and in place of oxalate, for obvious reasons.

The second method for the collection of blood is by capillary puncture. This is used mainly for sugar estimations. In some cases this method is inevitable, although experience points to the greater accuracy obtained by using larger amounts wherever possible. Thus capillary puncture frequently has to be resorted to in children, particularly if repeated observations have to be made. The method is also of value in extremely fat persons, where the veins may be both invisible and impalpable. If it be decided to adopt the capillary puncture method, the finger is generally selected.

The part chosen for the operation, usually the bed of the nail, is cleaned with ether, after the hand has been flushed by hanging in a dependent position, or by immersion in hot water. A sharp jab is given with a needle, preferably a flat surgical one, and the resulting drop of blood collected in a pipette. It is well to avoid, as much as possible, any squeezing or "milking" operation, since one tends to get tissue fluid in addition to blood.

Having obtained the blood, the next step is to decide on what particular technique to adopt for the analysis.

The methods come under two main headings :—

Firstly, *colorimetric* methods, which are rapid, easy and accurate, but expensive owing to the apparatus required ; and secondly, *volumetric*, which are also accurate, but require larger quantities of blood, and, although difficult and lengthy, have the advantage of being cheap from the point of view of apparatus. In the following description will be found representative techniques, all of which have been used personally by the authors, and have been found to be highly satisfactory.

In hospital practice, where large numbers of blood analyses have to be done, many of which are often urgent, we have found that Folin and Wu's system proves the most satisfactory.

In the following pages will be found the description of methods.

THE FOLIN AND WU SYSTEM OF BLOOD ANALYSIS

Use of the Colorimeter

There are two main types of colorimeter in use in this country. Firstly, the Dubosq pattern, and, secondly, the Klett Kober type.

From a wide experience of all colorimeters, we recommend the latter, since it is more accurate, and possesses divided mirrors, thus enabling the observer to adjust the

illumination with ease. The colorimeter must be kept scrupulously clean, and solutions must not be spilt over the mirrors. Although it is impossible to enter into a detailed account of colorimeters, the following description and diagram will help to give the reader some idea of how these instruments are constructed. The general arrangement of the colorimeter can be seen in the diagrammatic sketch given (see Fig. 45). It consists of an eye-piece giving an image of the contents of the cups. The light passes up in the paths shown by the dotted lines. It first passes through the solutions contained in the cups, up to the bottom of the plungers. Since these are made of glass, the light passes through them up to the prisms. These reflect the light as shown into the eye-piece. The image seen is shown below, and consists of

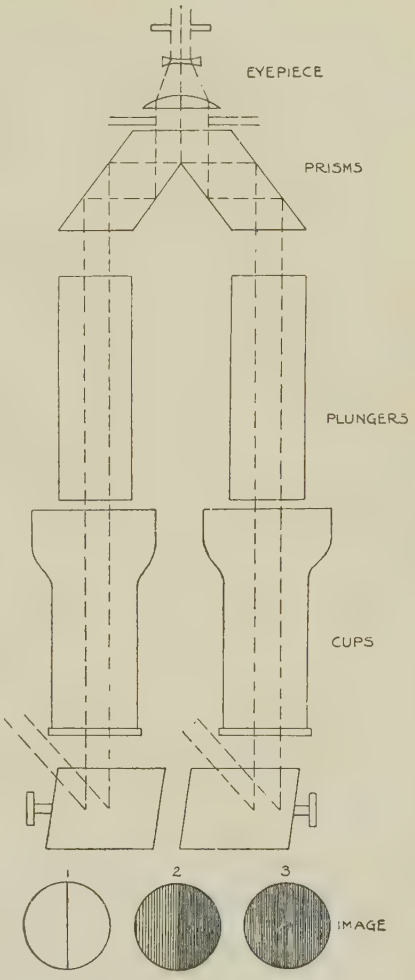


FIG. 45.—The Colorimeter.

1. Represents the appearance when the cups are empty.
2. When too great a depth is observed in the right cup.
3. Represents the match.

a circular field divided diametrically into two. One half represents the light transmitted through one cup and the other corresponds to that passing through the opposite cup. The cups are carried on platforms which can be moved vertically, and the distance between the bottom of the plunger and the bottom of the cup (*i.e.*, the depth of fluid observed) can be read off on a scale.

Whenever possible, it is advisable to use daylight as a source of illumination. The colorimeter is placed in a good and steady source of light, and the standard solution is poured into both cups, which are then adjusted so that the depth of fluid in each side is the same, usually 20 mm. The mirrors are then adjusted in such a manner that the colour on each side appears the same. The left-hand cup is then lowered, and the unknown solution is substituted for the standard. The colours are again matched by varying the depth of the unknown. It is essential to be sure that the scale reads at 0 when the plunger and bottom of cups are touching. If this be not so a zero correction must be applied in the case of the Dubosq instrument, whilst in the Kober there is an ingenious device for altering the zero. Three readings should always be taken, and with practice it will be found that they will be identical. Observations should consist in a series of rapid glances, and not a prolonged stare, otherwise fatigue and inaccuracy will result.

The process of calculation is extremely simple, provided that the depth of colour is directly proportional to the amount of substance present. This state of affairs usually prevails if there be not a great difference between the strength of the unknown and of the standard. No comparison should be attempted if the reading between the two differ by more than 20%. Either a stronger standard or weaker dilutions of the unknown should be prepared.

The amount of substance is given by the following calculation :—

$$\frac{\text{Depth of standard in millimetres}}{\text{Depth of unknown in millimetres}} \times \text{concentration of standard in mg. per c.c.}$$

This gives the concentration of the unknown in mg. per c.c. To convert the result obtained into mg. per 100 c.c. of blood all dilutions of the original blood will, of course, have to be allowed for.

THE METHODS OF FOLIN AND WU

Preparation of the Protein-free Filtrate

Solutions.—1. 10% sodium tungstate.[1] This should be quite clear, and alkaline to phenolphthalein. If not, the tungstate is not sufficiently pure for the purposes of blood analysis.

2. $\frac{2}{3}$ N. sulphuric acid.

Method.—Suitable pipettes are selected, and, if clean, are washed out with acetone and dried by air suction from a water pump, or by blowing air through by means of bellows. The following are then pipetted into a flask. The acid, which must be added last, is run in drop by drop, with gentle agitation of the contents of the flask.

One volume of blood.
Seven volumes of water.
One volume of the tungstate solution.
One volume of the acid.

The flask is then violently shaken, and if the precipitation of the blood proteins has been complete, the colour changes in a few minutes from bright red to a chocolate brown and there are no bubbles. If these conditions be satisfied the resulting solution is filtered, using the minimum quantity of filter paper and pouring the whole of the fluid on to the funnel at once. If not, the precipitation of proteins has been incomplete and the filtrate will be cloudy. The addition of 10% sulphuric acid, drop by drop, accompanied by vigorous

[1] Great care must be taken to ensure that all distilled water used in making up reagents, or during the analyses, is ammonia-free. No distinct colour should be given with Nessler's reagent.

shaking, may save the specimen, although this is not a desirable procedure. It is extremely important to remember to neutralise the filtrate treated in this manner before estimating urea by the soya bean method. The filtrate will be a water-clear fluid if all has gone well. This may be preserved almost indefinitely by the addition of a few drops of toluol, but the sugar will disappear more or less rapidly. According to Folin the addition of a drop of formalin to the blood prevents glycolysis and does not interfere with the subsequent determination. When dealing with cerebro-spinal fluid, it is recommended to use only a quarter volume of tungstate and sulphuric acid and to make up to the ten volumes with water. If serum is to be deproteinised, half volumes of tungstate and acid are sufficient.

Failures in the Preparation of a Protein-free Filtrate

The commonest causes of failure may be enumerated as follows :—

1. Inaccuracy in the preparation of the 10% sodium tungstate and $\frac{2}{3}$ N. sulphuric acid. If the sulphuric acid has been carelessly standardised, bad results will be obtained, owing to the acid being either too weak or too strong.

2. Too much potassium oxalate may have been added to the blood. This is one of the commonest causes of failure, and can be avoided by adhering to the method of preparing blood tubes already described.

A further word of warning must be given concerning the reaction of filtrates obtained by the addition of 10% sulphuric acid. These have a much lower *p*H than ordinary filtrates, and their reaction must be brought to the normal value. Ordinary filtrates just give a faint purple colour with bromcresol purple, *i.e.*, *p*H 6·5 to 6·8. If 10% acid has been added the reaction must be adjusted so that a faint purple colour is given with this indicator. The adjustment may be made by adding solid sodium carbonate and shaking.

Estimation of Non-protein Nitrogen

Solutions.—1. Digestion mixture.

50 c.c. of 5% copper sulphate solution.
300 c.c. of 85% phosphoric acid.
100 c.c. of nitrogen-free pure sulphuric acid.
450 c.c. distilled water.

2. Standard ammonium sulphate solution containing 1 mg. of nitrogen per 10 c.c. (0·4716 g. of specially purified ammonium sulphate per litre).

3. Nessler's solution. See under Blood Urea Estimation.

Method.—Into a hard glass test tube introduce the following :—

5 c.c. of blood filtrate.
1 c.c. of the acid digestion mixture and a quartz pebble.

Heat over a micro-burner or a sand bath and boil steadily. It is advisable to warm the whole tube first in order to avoid the danger of cracks. As the liquid concentrates it will darken, and when only about 1 c.c. remains dense white fumes will appear. When this stage is reached cover the mouth of the tube with a watch glass and cut down the flame slightly. Continue the heating until the liquid becomes clear and almost colourless. If brown, it is a sign that digestion is incomplete, and the combustion will have to be repeated. Allow to cool, add about 10 c.c. of water and immerse the tube in a boiling water bath. Apart from a slight opalescence the fluid should be colourless. This dissolves up most of the sulphates that have separated out. Filter whilst still hot into a 50 c.c. measuring flask, and extract with another 10 c.c. of water similarly. Cool the contents of the flask by immersion in cold water. If satisfactory hard glass tubes, such as Monax or Pyrex, can be obtained, these may be graduated at the 50 c.c mark, and the whole operation performed without filtration, etc. The

filtrate is digested in the tube, and after cooling, the contents are diluted with about 30 c.c. of distilled water. There should be no opalescence. Nesslerisation can then be performed in the tube.

Preparation of the Standard.—To a 100 c.c. measuring flask add 3 c.c. of the standard ammonium sulphate solution and 2 c.c. of the digestion mixture. Add about 60 c.c. of distilled water.

To the standard add 30 c.c. of Nessler's solution, and to the smaller flask add 15 c.c. Make up to the respective volumes, mix and compare in the colorimeter with the standard set at 20 mm.

Calculation.—Six hundred, divided by the colorimetric reading, gives the result in milligrams per 100 c.c.

Estimation of Blood Urea

Solutions.—1. Buffer solution.

69 g. monosodium phosphate.
179 g. crystallised disodium phosphate.
1 litre of distilled water.

2. Urease paper.

Thirty g. of powdered soya bean are ground up with 10 g. of permutit powder. The whole is well shaken with 120 c.c. of 16% alcohol. The resulting fluid is filtered through muslin, or a chardin paper, and strips of filter paper are drawn through the filtrate, after which they are hung up to dry. The dry paper is then cut up into pieces about an inch square. One suffices for a determination.

3. Nessler's reagent. Ordinary Nessler's reagent is unsuitable, and must not be used. The following method of preparation must be adhered to :—

150 g. potassium iodide.
110 g. iodine.
140 to 150 g. metallic mercury.
100 c.c. water.

These are all introduced into a 500 c.c. flask, which is shaken vigorously for seven to fifteen minutes. When the solution, which is at first red, begins to pale, cool the contents of the flask by immersion in cold water. After a short time the red is replaced by the green colour of the double iodide. When this stage has been reached decant the green solution into a two-litre measure. Wash the mercury left in the original flask with several quantities of water, pouring the washings into the two-litre measure. Make up the volume with distilled water. To make the final solution add 750 c.c. of this solution to 3,500 c.c. of 10% NaOH (made by diluting a clear saturated solution, containing about 55 g per 100 c.c., and checking by titration ; an accuracy of 5% is necessary), and the whole must be diluted to 5 litres. The final Nessler's solution should be quite clear and ready for use. If cloudy, allow to stand for some time, when the clear supernatant fluid can be decanted.

4. Saturated solution of borax.

5. $\frac{N}{20}$ hydrochloric acid.

Method.—Into a hard glass test tube (1 by 8 inches), add the following :—

5 c.c. filtrate.
2 drops of buffer solution.
1 piece of urease paper.

Incubate in a water bath at 55° C. for eight to ten minutes. Remove from bath and add 2 c.c. of the saturated borax solution. The urea nitrogen has now been converted into ammonia, whilst the addition of borax renders the solution sufficiently alkaline to enable the ammonia to be distilled off. If the solution be heated, it will froth violently, rendering distillation impossible. The addition of paraffin is of little avail, whilst caprylic alcohol merely distils over and causes a precipitation when Nessler's solution is added in the final stage. After many experiments, it was found that if a wire spring be placed in the tube the bubbles break up

and distillation is rendered easy. Experience has shown that aeration methods are not so reliable as distillation.

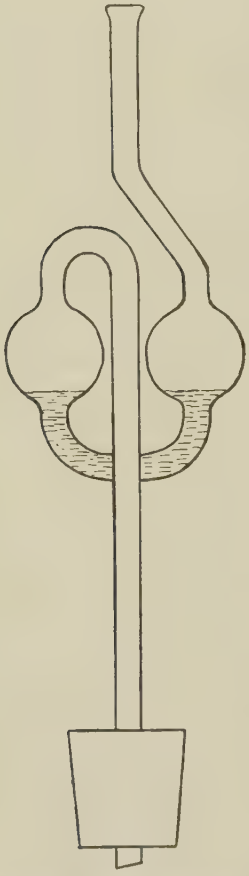


Fig. 46.—Absorption Bulb-tube used in the determination of blood urea.

Insert a spiral spring of wire in such a manner that it covers practically all the inner surface of the tube. Close the mouth of the test tube with a rubber stopper bearing a bulb absorption tube (see Fig. 46), containing 2 c.c. of $\frac{N}{20}$ hydrochloric acid. The diameter of the bulb should be 1 inch and the tubing should be of such a size that the 2 c.c. of acid completely seal off the connection between the bulbs. The base of the large tube is carefully heated with a micro-burner or on a sand-bath until the contents begin to boil. The distillation is continued until the contents of the bulbs begin to boil and steam issues from the open top. The bulb-tube is removed and its contents are washed into a test tube, graduated at 25 c.c. with distilled water. The final volume should not exceed 22 c.c. and should be cooled prior to the addition of Nessler's reagent.

Preparation of the Standard.—Three c.c. of the standard ammonium sulphate solution, referred to under the estimation of non-protein nitrogen, are measured into a 100 c.c. measuring flask, and about 80 c.c. of distilled water are added.

Ten c.c. of Nessler's solution are added to the standard.

and 2·5 c.c. to the tube containing the distillate. The volumes are then made up to 100 c.c. and 25 c.c. respectively. After thorough mixing they are compared in the colorimeter with the standard at 20 mm.

Calculation.—Let the colorimetric reading of the unknown be R.

Then, $\frac{20 \times 15 \times 15}{R \times 7} = \frac{642{\cdot}8}{R}$ = milligrams of urea per 100 c.c.

The commonest causes of failure in this determination are as follows :—

1. Excessive frothing during the distillation. This is usually prevented by the spiral spring of wire. Folin suggests the addition of 2 drops of liquid paraffin to the solution before the heating is commenced.

2. Turbidity of the final solution to be compared. The commonest cause of this is insufficient cooling of the solutions before the addition of Nessler's solution. If the urea content be high, 200 mg. per 100 c.c. or over, a precipitate is almost sure to form and render accurate comparison impossible. This difficulty can be averted by taking a smaller volume of blood filtrate, say, 2·5 or even 1 c.c.

3. Incomplete hydrolysis of the urea, thus giving too low a reading. This usually happens when 10% acid has been added to render the precipitation of the original blood complete. This can be averted by the addition of a pinch of solid sodium carbonate to neutralise the excess acidity, as outlined under the section on " Failures in the Preparation of a Protein-free Filtrate."

Estimation of Uric Acid

Solutions.—1. Uric acid reagent.

The following formula for making up this reagent was given to the authors in a letter from Professor Folin :—

Fifty c.c. of 85% phosphoric acid, sp. gr. 1·75, and 160 c.c.

of water are introduced into a flask which is heated until the contents boil, when 100 g. of sodium tungstate are added. It is essential to use phosphoric acid of the strength prescribed, otherwise some of the lithium carbonate will not go into solution, and a defective reagent will result. The boiling is continued gently for one hour, using a micro-burner as the source of heat, and a funnel in the mouth of the flask as a condenser.

Twenty-five g. of lithium carbonate are transferred to a beaker and 50 c.c. of 85% phosphoric acid are added.

Two hundred c.c. of water are then added and the contents of the beaker are boiled in order to drive off CO_2. The first solution is then added to this and the whole is made up to 1 litre.

2. 15% sodium cyanide in decinormal NaOH.
3. 20% solution of lithium sulphate.
4. Uric acid standard.

To 0·2 g. of uric acid in a beaker add 20 c.c. of a 0·5% solution of lithium carbonate. It is advisable to add the lithium carbonate solution at a temperature of about 60° C. or 70° C. Stir until all the uric acid has dissolved. Add 10 c.c. of water, 10 c.c. of 40% formalin, and 2 c.c. of 50% acetic acid. Transfer the resulting solution to a 200 c.c. measuring flask and make up to the mark with distilled water. This solution contains 1 mg. of uric acid per cubic centimetre. From this stock solution the urine standard can be prepared by making a 1 in 10 dilution, and the blood standard by making a 1 in 250 dilution. To prepare the blood standard, therefore, one proceeds as follows :—

To a 250 c.c. measuring flask 1 c.c. of the stock solution is added, together with about 100 c.c. of water and 10 c.c. of $\frac{2}{3}$ N. sulphuric acid. One c.c. of 40% formalin is then added and the volume is made up to the mark with water.

Method.—Five c.c. of uric acid solution and 5 c.c. of blood filtrate are set up in test tubes graduated at 25 c.c. One c.c. of uric acid reagent, 2 to 4 drops of lithium

sulphate solution, 2 c.c. of water and 2 c.c. of the cyanide solution are added to each. After standing at room temperature for two minutes, the tubes are immersed in a boiling water bath for one minute. After cooling and diluting to 25 c.c. the resulting blue solutions are compared in the colorimeter, the standard being set at 20 mm. The comparisons must be made at once, otherwise fading may occur.

Calculation.—Eighty, divided by the colorimetric reading, gives the uric acid content in milligrams per 100 c.c.

Estimation of Creatinin

Solutions.—1. Standard creatinin solutions: 1·61 g. of the creatinin zinc chloride compound is dissolved in sufficient $\frac{N}{10}$ HCl to make a litre. The creatinin zinc chloride compound may be bought, or it can be made from urine. This solution will contain 1 mg. creatinin per c.c. This stock solution is suitable for the determination of urinary creatinin. The blood standard is prepared by diluting 6 c.c. to 1 litre, after the addition of 10 c.c. of decinormal HCl.

2. Saturated aqueous solution of purified picric acid. It is essential that purified picric acid be used. This may be obtained by the method given by Folin and Doisy (6) or by Benedict (7).

3. 10% NaOH.

4. Alkaline picrate solution. This must be freshly prepared each time. It is made by mixing saturated picric acid solution and 10% NaOH in the proportions of 5 to 1 respectively.

Method.—Put up the unknown and standard as follows in tubes graduated at 15 and 30 c.c. :—

Unknown.	*Standard.*
10 c.c. filtrate.	5 c.c. standard creatinin solution.
5 c.c. alkaline picrate.	15 c.c. water.
	10 c.c. alkaline picrate.

Let stand for eight minutes.

Compare in the colorimeter with standard at 20 mm.

Calculation.—Thirty, divided by the colorimetric reading, gives the creatinin content in milligrams per 100 c.c.

Estimation of Amino-acid Nitrogen in Blood

Solutions.—1. Standard solution of an amino acid. Almost any amino acid will do, but generally it will be found that glycine is the most convenient. A solution of glycine is made by dissolving 0·0536 g. of pure acid in 100 c.c. of decinormal HCl containing 0·2% of benzoic acid. From this stock solution, which contains 0·1 mg. of nitrogen per c.c., the actual standard is prepared by diluting 70 c.c. to 100 c.c. with decinormal HCl. The resulting solution contains 0·07 mg. of nitrogen per c.c.

2. Sodium carbonate solution. Fifty c.c. of saturated sodium carbonate solution are diluted to 500 c.c. with water. By titration with decinormal HCl, using methyl red as an indicator, and subsequent dilution, the strength of the carbonate solution is so adjusted that 8·5 c.c. are neutralised by 20 c.c. of the acid.

3. Acetic acid solution.

This is prepared by diluting 100 c.c. of 50% acetic acid with an equal volume of 5% sodium acetate solution.

4. A 4% solution of sodium thiosulphate ($Na_2S_2O_3.5H_2O$).

5. Fresh 0·5% solution of the sodium salt of β-naphthoquinone-sulphonic acid. Since this substance can now be bought, its preparation will not be described. The preparation is described by Folin (4).

Tests of Purity for the Salt.—1. A 1% solution of the salt set up in one of the cups of a colorimeter is compared with a 0·5 N. potassium bichromate solution. When the depth of the bichromate solution is 20 mm. the quinone solution should read off at 26 to 27 mm.

2. Tests for ammonia and coloured decomposition products are given in Folin's original paper (4), which should be

consulted if any doubt as to the purity of the compound arise.

Method.—Put up two graduated tubes as follows :—

Standard.	*Filtrate.*
1 c.c. of standard glycine solution.	5 c.c. of filtrate.
3 c.c. of water.	2 drops of 0·25% alcoholic phenolphthalein.
2 drops of 0·25% alcoholic phenolphthalein.	Enough carbonate to produce the same colour as in the standard.
1 c.c. of special sodium carbonate solution.	
5 c.c. of water.	

Add 1 c.c. of freshly prepared naphthoquinone solution to the filtrate and 2 c.c. to the standard.

Allow to stand in the dark for twenty to thirty hours.

Add 2 c.c. of the acetic acid solution to the standard and 1 c.c. to the filtrate. Shake and add thiosulphate solution, 2 c.c. to the standard and 1 c.c. to the filtrate. Make the standard up to 30 c.c., the unknown to 15 c.c. Compare with the standard at 20 mm.

Calculation.—One hundred and forty, divided by the colorimetric reading, gives the amino-acid nitrogen content in milligrams per 100 c.c.

Estimation of Blood Chlorides (van Slyke (8))

Solutions.—1. $\frac{N}{10}$ silver nitrate. Dissolve 8·495 g. $AgNO_3$ in a minimum amount of water and dilute to 1 litre with nitric acid (sp. gr. 1·4).

2. $\frac{N}{50}$ ammonium thiocyanate.

3. Five per cent. ferric alum in water.

Method.—Introduce 1 c.c. of oxalated or citrated blood in a 100 c.c. hard glass test tube and add 3 c.c. $\frac{N}{20}$ silver nitrate solution. Cover with a watch glass and heat in a water bath till the solution above the silver chloride precipitate is clear and light yellow in colour. Cool, add 6 c.c. ferric alum solution and titrate the excess silver with the standard thiocyanate until a definite salmon pink colour is obtained.

Calculation.—117 × (7·5 minus c.c. $\frac{N}{50}$ thiocyanate) = mg. NaCl per 100 c.c. blood. Control titrations are made in which 3 c.c. of the silver nitrate solution, after adding 6 c.c. ferric alum solution, are titrated directly with $\frac{N}{50}$ thiocyanate. If the latter vary from 7·5 c.c. the thiocyanate figure in the calculation is to be multiplied by $\frac{7{\cdot}5}{\text{c.c. thiocyanate used in control}}$.

Estimation of Blood Sugar

Solutions.—1. Standard sugar solutions.

One g. of pure glucose is dissolved in 50 c.c. of 0·25% benzoic acid solution in water. This solution is then transferred to a 100 c.c. volumetric flask and made up to the mark with benzoic acid solution. One c.c. of this solution made up to 100 c.c. will serve as a standard for most bloods, but it is well to have a double standard ready in case a very high blood sugar is encountered. This is made by diluting 2 c.c. of the stock solution to 100 c.c. (all dilutions are made with saturated benzoic acid). These solutions contain 0·1 and 0·2 mg. of glucose per c.c.

2. Alkaline copper solution.

Forty g. of anhydrous sodium carbonate are dissolved in

400 c.c. of water, and the resulting solution is transferred to a litre flask. 7·5 g. of tartaric acid are added, and when this has dissolved, 4·5 g. of crystalline copper sulphate are added. After thorough shaking, the substances dissolve and the volume is made up to a litre.

3. Molybdic acid solution.

Thirty-five g. of molybdic acid and 5 g. of sodium tungstate are added to a litre beaker, together with 200 c.c. of 10% NaOH and 200 c.c. of water. The contents of the beaker are then boiled for half an hour or so in order to remove the ammonia from the molybdic acid. After cooling, the solution is transferred to a 500 c.c. measuring flask, and diluted up to about 350 c.c. One hundred and twenty-five c.c. of 85% phosphoric acid are then added and the volume is made up to 500 c.c.

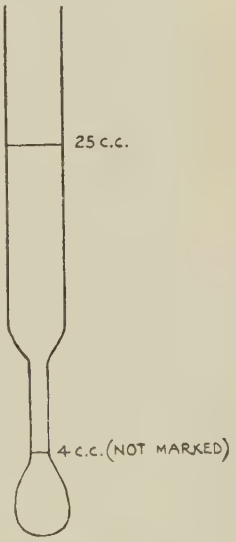


FIG. 47.—Folin's Blood-sugar Tube.

Method.—Two c.c. of filtrate and 2 c.c. of standard are transferred to special sugar tubes (see Fig. 47).

Two c.c. of the alkaline copper solution are added to each, and the tubes are immersed in a boiling water bath for six minutes. They are then removed and cooled in water. Two c.c. of molybdic acid are added to each, and after shaking, the volume is made up to the 25 c.c. mark with water. After mixing, the solutions are compared in the colorimeter, the standard being set at 20 mm. If there be any reason to suspect that the blood sugar content is likely to be high it is as well to employ both standards.

Calculation.—2,000 for the low standard, and 4,000 for the high standard, divided by the colorimetric reading, gives the sugar content in milligrams per 100 c.c.

The importance of the remarks on p. 375 concerning glycolysis must never be lost sight of. This is particularly so when a number of blood sugar estimations have to be performed, as in the case of sugar tolerance tests. The filtrate should be prepared from each specimen of blood as it is collected, and on no account should they be kept until the test is finished.

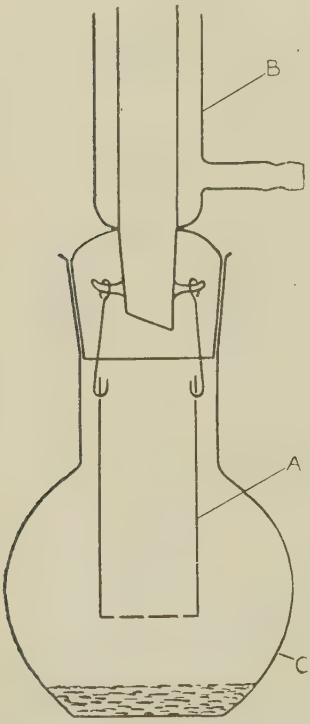


Fig. 48.—Modified Extraction Apparatus used in the Determination of Blood Cholesterol Content.

Estimation of Blood Cholesterol

Method of Myers and Wardell (9)

One c.c. of blood, plasma or serum is mixed intimately with about 5 g. of plaster of Paris in a porcelain dish. The mixture is dried in an air oven at 100° C. for two hours or left over-night in a desiccator. The contents of the dish are then finely powdered and transferred to a filter paper extraction thimble which is placed in a short glass tube (A), the bottom of which is perforated with a number of holes. This is now attached by means of wire hooks to the condenser (B) of the extraction apparatus and the flask (C), containing about 20 c.c. of chloroform, is placed in position (see Fig. 48). Extraction is continued for about ninety minutes on a boiling water bath or an electric hot plate.

The chloroform extract is transferred to a tube and made

up to 15 c.c., and 15 c.c. of a standard cholesterol solution in chloroform containing 0·1 mg. per c.c. are measured into a similar tube. Four c.c. of acetic anhydride, and 0·4 c.c. of pure sulphuric acid are added to each solution. After thorough mixing the tubes are transferred to a dark cupboard for ten minutes in order to allow the colour to develop. The green tints are then compared in a colorimeter, with the standard at 20 mm.

Calculation.—$\frac{20}{\text{reading}} \times 1{\cdot}5 \times 100 =$ cholesterol content in milligrams per 100 c.c. The normal values, as obtained by this method, vary from 150 to 180 mg. per 100 c.c. for serum, and from 140 to 170 mg. per 100 c.c. for whole blood.

Estimation of the Phosphate Content of Plasma

This method, as recommended by de Wesselow (10), includes the improvements of the following workers : Bell and Doisy (11), Lehman (12), Dennis and von Meysenbug (13) and Tolstoi (14).

Solutions.—1. Stock solution of monopotassium phosphate. The pure salt is powdered and desiccated for several days over sulphuric acid. Of this preparation 4·394 g. are dissolved and made up to 1 litre with phosphate-free water in an accurate flask.

This solution contains 1 mg. of phosphorus in 1 c.c. For the actual determination a weaker solution, containing 0·05 mg. of phosphate in 10 c.c., is used. To prepare this 5 c.c. of the strong standard solution are made up to 1 litre.

To avoid the growth of moulds the flasks are kept tightly stoppered and a small quantity of chloroform may be added.

2. Carbonate sulphite solution. To 2 litres of a 20% solution of sodium carbonate 75 g. of sodium sulphite, dissolved in 500 c.c. of water, are added. The solution is then

filtered. This solution should be made up in small quantities, since its keeping properties are poor.

3. Trichloracetic acid solution. Twenty per cent. solution of trichloracetic acid.

4. Molybdic acid solution. One hundred g. of pure ammonium molybdate are dissolved without heat in 1 litre of phosphate-free $2N.H_2SO_4$. Five c.c. of this solution are treated with an equal amount of the hydroquinone solution. After standing for five minutes, 25 c.c. of the carbonate sulphite solution are added. If the reagents be satisfactory the resulting mixture should be absolutely colourless. If this test be unsatisfactory a fresh sample of ammonium molybdate should be used, or it may be purified by the method given by Bell and Doisy (11). This solution keeps indefinitely, a little precipitation being of no importance.

5. Hydroquinone solution. Twenty g. of pure hydroquinone are dissolved in 1 litre of phosphate-free water, and then 1 c.c. of concentrated sulphuric acid is added.

On standing this solution becomes coloured, owing to the formation of quinone. It may be used as long as the coloration is moderate, since the quinone is reduced in the determination of the phosphates by the alkaline sulphite solution. This solution should be kept in a tightly stoppered bottle to prevent oxidation.

Method.—Five c.c. of plasma (or 2 c.c. if the phosphate content be suspected to be high) are run into a 25 c.c. flask containing 10 c.c. of water and 5 c.c. of the trichloracetic acid solution. After shaking, make up to the mark with water and mix. On standing for ten minutes, filter through a filter paper which has been well washed with slightly acidified water and dried. This removes any trace of phosphate from the paper.

To 10 c.c. of perfectly clear trichloracetic acid filtrate in a 25 c.c. flask, add 1 c.c. of molybdic acid solution and 2 c.c. of the hydroquinone solution. At the same time similar quantities of molybdic acid and hydroquinone are added to

a 25 c.c. flask containing 10 c.c. of the weak standard phosphate solution (0·05 mg. P) and 2 c.c. of trichloracetic acid. After five minutes 10 c.c. of the carbonate sulphite solution are added to each flask, and the flask containing the plasma filtrate is made up to the 25 c.c. mark with water. Compare in the colorimeter after standing for 10 minutes.

If the standard cup be set at 20, and a 1 in 5 dilution of plasma used, then $\frac{50}{\text{reading}}$ = mg. P per 100 c.c. of plasma.

Thus, with a reading of 15 for the plasma, each 100 c.c. of plasma would contain 3·3 mg. of inorganic phosphate expressed as phosphorus.

It may be necessary to use a stronger standard or less filtrate with bloods rich in phosphates.

Estimation of Blood Proteins (Hawk and Bergeim (15))

Total Proteins.—Dilute 1 c.c. of plasma or serum to 50 c.c. with 0·9% sodium chloride. Using 1 c.c. of this solution proceed as under "Digestion."

Fibrinogen.—To 1 c.c. of plasma add 48 c.c. of 0·9% sodium chloride and 1 c.c. of 2·5% calcium chloride. Mix and allow to stand twenty minutes. The fibrin clot may be broken with a glass rod and the solution filtered. One c.c. of the solution is used in the digestion.

Albumin.—To 1 c.c. of plasma or serum add exactly 30 c.c. of 22·2% sodium sulphate which has been warmed to 37° C. Stopper the flask and allow to stand at 37° C. for three hours. Filter through a fine filter paper and use 1 c.c. of the clear filtrate for the digestion.

Digestion.—Into a resistance glass test tube (Monax or Pyrex), 200 mm. × 25 mm., introduce 1 c.c. of the solution prepared as above and 1 c.c. of the digestion mixture used

in the determination of non-protein nitrogen (see p. 381). Add a chip of broken porcelain and one drop of caprylic alcohol. Heat with a microburner or on a sand bath until all the water has been boiled off and the contents char. Cool for thirty seconds and add carefully, one drop at a time, 30% hydrogen peroxide until the solution clears, recording the number of drops used. Boil again for one minute. Cool and dilute to 35 c.c. with water.

Into a 100 c.c. flask introduce 3 c.c. of the standard ammonium sulphate solution (containing 0·1 mg. of nitrogen per 1 c.c., see p. 381) and 2 c.c. of the acid digestion mixture. Dilute to about 70 c.c. To the standard add 30 c.c. and to the unknown 15 c.c. of Nessler's solution and compare in the colorimeter with the standard set at 20 mm.

A blank determination on the hydrogen peroxide should be carried out by adding 30 drops of the peroxide to 1 c.c. of the boiling digestion mixture. After the digestion is completed the nitrogen equivalent of one drop of the peroxide is obtained by diluting, adding Nessler's solution and comparing with the standard as above.

Calculation.—The protein values are calculated from the nitrogen values by multiplying by 6·25. A correction for the nitrogen content of the peroxide is made by subtracting from the total nitrogen of the sample, the nitrogen equivalent per drop (E) times the number of drops used (n).

Protein in g. per 100 c.c. =

$$\left[\left(\frac{Rs}{Rv} \times 0{\cdot}15 - nE\right)\frac{100}{V} - N.P.N.\right] \times \frac{6{\cdot}25}{1{,}000}$$

in which Rs and Rv represent the readings of the standard and the unknown; n and E have the significance above described; N.P.N. represents the non-protein nitrogen in mg. per 100 c.c. For total protein and protein in the fibrinogen filtrate, V = 0·02; for albumin, V = 0·0323 $\left(i.e., \frac{1}{31}\right)$.

Fibrinogen = Total protein minus protein in fibrinogen filtrate.

Globulin = Total protein minus albumin minus fibrinogen, in plasma, since fibrinogen is absent from serum.

Colorimetric Estimation of Hæmoglobin (Myers (16))

The specimen of oxalated or citrated blood is well shaken up to ensure thorough mixing of the cells and plasma. 0·025 c.c., or 0·05 c.c. if it be suspected that the hæmoglobin is very low, is pipetted into a test tube containing 10 c.c. of $\frac{N}{10}$ hydrochloric acid; great care should be taken to wash out the pipette with the acid. The mixture is allowed to stand for two hours as it has been shown that the full colour of the acid hæmatin does not develop until this period has elapsed. The colour of this solution is then matched to a standard glass plate (Newcomer), which is inserted in the top of one of the plungers of the colorimeter. After the standard plate has been inserted, the cup corresponding to that plunger is filled with water and is adjusted to the 10 mm. mark. The unknown solution of acid hæmatin, referred to above, is then placed in the second cup and is matched against the standard glass plate. Slight difficulty may be experienced owing to the fact that the unknown solution is slightly turbid whereas the glass plate is clear. A little experience, however, enables accurate comparisons to be made.

Calculation.—It is assumed that the plate corresponds with 0·038% of hæmoglobin. The hæmoglobin content of the blood is given by the following calculation:—

$$\frac{10}{R} \times 0{\cdot}038 \times \text{dilution} = \text{g. hæmoglobin per 100 c.c.}$$

It will be seen that by this method the hæmoglobin content is given in g. per 100 c.c. and not in the traditional

manner as a percentage of an arbitrary normal. One hundred per cent. hæmoglobin on Haldane's scale corresponds with 13·8 g. of hæmoglobin per 100 c.c. blood. This is regarded by many workers, particularly those in America, as being too low. The normal standard is variously given as 15·6 to 16·92 g. per 100 c.c. As has been pointed out by many, it is obviously preferable to record the hæmoglobin in g. per 100 c.c., but owing to the fact that all published work in this country is worked out on the Haldane scale, it would be necessary to convert readings given by the above method to that of Haldane. This can be done very easily by taking Haldane's standard, namely, 13·8 g. per 100 c.c. as 100%, and calculating the percentage deviation of the figure found by the colorimetric method.

Normally the total solids of blood are in the region of 21%, and hæmoglobin is in the region of 16%. It follows, therefore, that the estimation of the hæmoglobin content may be used in place of the estimation of total solids for the purpose of determining the dilution or concentration of the blood.

Estimation of Icterus Index (Bernheim (17))

For this determination clear, non-hæmolysed serum or plasma should be used. This is compared in the colorimeter against a standard solution of potassium dichromate, set at 15 mm. The strength of the solution is 1 in 10,000, *i.e.*, 50 mg. of potassium dichromate to 50 c.c. water. Should the serum be too deeply tinted with bile, it may be diluted with normal saline.

Calculation.—$\frac{15}{R} \times$ dilution (if any) gives the icterus index.

The normal range is from 4 to 6. The method is very useful in following a patient from week to week in order to find out whether the jaundice is increasing or decreasing. The estimation may be used in place of the quantitative

van den Bergh reaction described on p. 155. There are, however, several sources of error, such as staining of the serum due to pigments other than bile, cloudiness of the serum, or fading of the standard solution. The latter may be avoided by keeping the dichromate standard in the dark. The test serves as a useful rough, clinical guide to the progression or retrogression of jaundice.

NON-COLORIMETRIC METHODS

Estimation of Blood Urea (Marshall (18), Van Slyke and Cullen (19) and MacLean (20))

Solutions.—1. $\frac{N}{100}$ sulphuric acid.

2. $\frac{N}{100}$ sodium hydroxide.
3. A saturated solution of methyl red in 50% alcohol.
4. A solution containing 6 g. of potassium hydrogen phosphate in 1 litre of water.
5. Ten g. of soya bean in 100 c.c. water are allowed to stand with occasional shaking for one hour. Ten c.c. $\frac{N}{10}$ hydrochloric acid are added and the mixture is allowed to stand for fifteen minutes longer. It is now filtered and a few drops of toluene are added. This solution can be used for at least five days after its preparation.
6. Solid anhydrous potassium carbonate.

Method.—Three c.c. of oxalated blood are run into tube B (see Fig. 49) containing 3 c.c. of the phosphate solution. Six drops of caprylic alcohol and 1 c.c. of 10% soya bean extract are added, and the tube is then stoppered and is placed in a water bath at a temperature of 42° C. for fifteen minutes. By this means the urea is hydrolysed by the urease into ammonia and carbon dioxide.

While digestion is in progress, measure out 25 c.c. $\frac{N}{100}$ acid into another tube A, together with 2 drops of methyl red and 1 drop of caprylic alcohol. When the decomposition of the

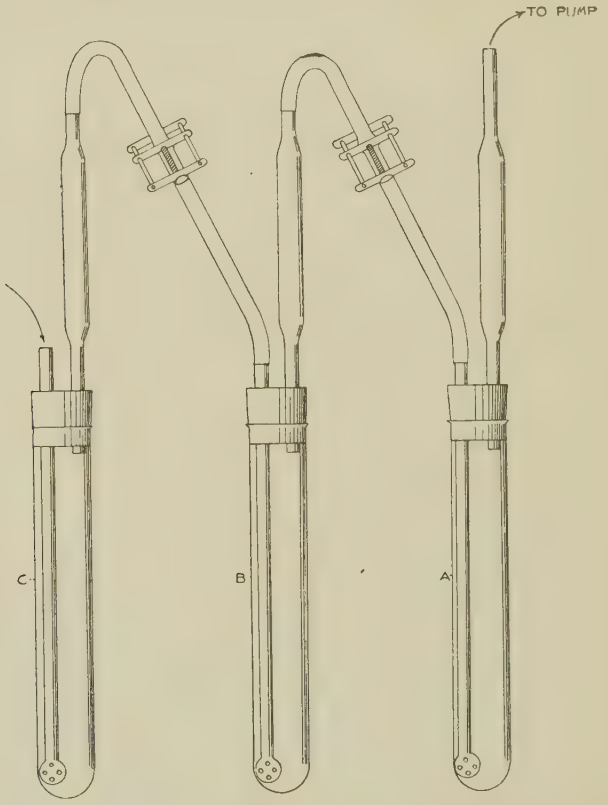


FIG. 49.—Apparatus for the Estimation of Urea and Non-protein Nitrogen in Blood.

urea is complete, the tube is removed from the water bath and connected on one side with tube A, which contains the 25 c.c. $\frac{N}{100}$ acid, and on the other side with tube C containing,

some 5% sulphuric acid, to absorb any ammonia in the aspirated air. Tube A is then connected to a suction pump. The clips are now opened and the air current is passed through for one minute, in order to carry over into tube A any traces of ammonia that may have escaped into the air space of tube B during decomposition. The suction is stopped and the blood tube B is opened and 4 to 5 g. of potassium carbonate are added. The cork is replaced immediately and the air current is now passed through slowly at first, but after a few minutes the pump is turned on to its fullest capacity. The time required for complete aeration depends on the pump used, but with ordinary power the experiment should be complete in thirty to forty minutes. The tube A containing the standard acid is now disconnected, and the excess acid therein is titrated with $\frac{N}{100}$ NaOH until the indicator gives a faint buff colour. The difference between the 25 c.c. acid originally taken and the number of c.c. of alkali used gives the number of c.c. neutralised by the ammonia.

Calculation.—This is very simple, since each c.c. of $\frac{N}{100}$ acid neutralised equals 10 mg. urea per 100 c.c. blood. Grams urea per 100 c.c. blood $= 0{\cdot}01 \times$ c.c. $\frac{N}{100}$ acid. Urea nitrogen per 100 c.c. blood $= 0{\cdot}00466 \times$ c.c. $\frac{N}{100}$ acid. In the calculation it is usual to subtract 0·4 c.c. from the acid neutralised in order to allow for the ammonia in the reagents.

Estimation of Non-Protein Nitrogen (MacLean (20))

Preparation of Blood Filtrate.—Three c.c. of oxalated blood are pipetted into a flask containing 12 c.c. distilled water, and then 15 c.c. of a 10% trichloracetic acid solution are added. After thoroughly mixing, and standing for half

an hour, the contents of the flask are filtered. The filtrate should be perfectly clear.

Method.—Ten c.c. of the above filtrate are measured into a hard, heat-resisting glass test tube B (Fig. 49), together with 1 c.c. of pure concentrated sulphuric acid and 3 drops of a 5% copper sulphate solution. A few very small beads and 0·1 g. finely ground pumice are added to prevent bumping. The tube is then placed in a slanting position and the mixture boiled down over a very small flame to a volume of 3 c.c. The tube is then restored to an upright position and heated until the contents begin to char. At this stage 0·3 g. of potassium sulphate is added and heating is continued for fifteen minutes after the mixture clears. While still warm 6 c.c. of distilled water are added and the tube is cooled under the tap. The distillation of ammonia may now be performed. An apparatus exactly similar to that described for the urea estimation is used (see Fig. 49), liberation of ammonia being accomplished by the addition of 3 c.c. saturated potassium hydroxide. To prevent any escape of ammonia during the addition of alkali, an inner tube, graduated to contain 3 c.c. up to the mark, is used. By sucking up the caustic potash solution to the mark and closing the rubber tube attached, the alkali remains in this tube until the clip is released. The rubber attached to the short tube is now closed with a clip, and the inner tube, containing the potash, inserted into the larger tube. The rubber tubes are now attached to a tube containing a 5% solution of sulphuric acid on one side and to one containing 25 c.c. of a standard solution of $\frac{N}{100}$ sulphuric acid on the other. The suction pump is attached to the tube with the standard acid. The clip on the short tube is now opened and the potash allowed to mix with the solution in the large tube by opening the clip attached to the inner tube. The air current is passed through, slowly at first, and after a few minutes the pump is turned on to its fullest capacity for

thirty minutes. Disconnect and titrate with $\frac{N}{100}$ NaOH.

Calculation.—A blank equivalent to 0·15 c.c. $\frac{N}{100}$ H_2SO_4 should be allowed for the nitrogen contained in the chemicals used. One c.c. $\frac{N}{100}$ H_2SO_4 = 0·00014 g. nitrogen.

Micro-estimation of Blood Sugar by the Method of Hagedorn and Jensen (21)

Solutions :—

1. Caustic soda, $\frac{N}{10}$.
2. Zinc sulphate, 0·45%.
3. Potassium ferricyanide, 1·65 g.
   Sodium carbonate (anhydrous and freshly heated), 10·6 g.
   Water to 1,000 c.c.
   This solution must be protected from light.
4. Potassium iodide, 25 g.
   Zinc sulphate, 50 g.
   Sodium chloride, 250 g.
   Water to 1,000 c.c.
5. Glacial acetic acid (Fe-free), 3 c.c.
   Water to 100 c.c.
6. 1 g. soluble starch in 100 c.c. saturated NaCl solution.
7. Sodium thiosulphate solution, $\frac{N}{200}$.
8. Potassium iodate solution, $\frac{N}{200}$, prepared by dissolving 0·3566 g. dry KIO_3 in 2 litres water.
   This solution must be accurate.

Method.—In a test tube (15 × 150 mm.), 1 c.c. $\frac{N}{10}$

caustic soda is mixed with 5 c.c. 0·45% zinc sulphate. This forms a gelatinous precipitate of zinc hydroxide.

0·1 c.c. blood is now measured in a capillary pipette, blown into the mixture and the pipette quickly washed out with the solution. The tube is now heated in a boiling water bath for three minutes and the contents filtered through a small wad of washed cotton wool into a test tube. The filter is washed twice with 3 c.c. of water. After the addition of 2·0 c.c. of the alkaline ferricyanide solution the tube is heated for fifteen minutes in a boiling water bath.

After cooling, 3 c.c. of the iodide-zinc sulphate solution and 2 c.c. acetic acid solution are added, and the solution titrated with the thiosulphate solution, using 2 drops of the starch solution as an indicator.

The thiosulphate is added from a micro-burette which contains 2 c.c. and is graduated to 0·02 c.c.

A blank value is also obtained by carrying out the whole determination without the addition of blood. The thiosulphate is checked by titration of 2 c.c. of the $\frac{N}{200}$ potassium iodate to which has been added 3 c.c. $KI - ZnSO_4$ solution and 2 c.c. acetic acid. Then 2 divided by this reading gives the factor F for the thiosulphate. Let A be the thiosulphate burette reading for the unknown and B be that for the blank. The glucose values of A multiplied by F c.c. thiosulphate and B multiplied by F c.c. thiosulphate are obtained from the table on p. 405. The difference between these values gives mg. glucose in 0·1 c.c. of blood.

The results may also be calculated as follows :—

$$2 - F(A - B) = \text{c.c. } \frac{N}{200} \text{ ferricyanide reduced.}$$

Two c.c. ferricyanide are reduced by 0·385 mg. glucose.

Therefore mg. glucose per 100 c.c. of blood =

$$\left[\frac{0{\cdot}385 \times 2 - F(A - B)}{2}\right] \times 1{,}000.$$

Table. (Hagedorn and Jensen.)

c.c. $\frac{N}{200}$ $Na_2S_2O_3$—mg. Glucose.

	0·00	0·01	0·02	0·03	0·04	0·05	0·06	0·07	0·08	0·09
0·0	·385	·382	·379	·376	·373	·370	·367	·364	·361	·358
0·1	·355	·352	·350	·348	·345	·343	·341	·338	·336	·333
0·2	·331	·329	·327	·325	·323	·321	·318	·316	·314	·312
0·3	·310	·308	·306	·304	·302	·300	·298	·296	·294	·292
0·4	·290	·288	·286	·284	·282	·280	·278	·276	·274	·272
0·5	·270	·268	·266	·264	·262	·260	·259	·257	·255	·253
0·6	·251	·249	·247	·245	·243	·241	·240	·238	·236	·234
0·7	·232	·230	·228	·226	·224	·222	·221	·219	·217	·215
0·8	·213	·211	·209	·208	·206	·204	·202	·200	·199	·197
0·9	·195	·193	·191	·190	·188	·186	·184	·182	·181	·179

Table. (Hagedorn and Jensen)—*continued.*

	0·00	0·01	0·02	0·03	0·04	0·05	0·06	0·07	0·08	0·09
1·0	·177	·175	·173	·172	·170	·168	·166	·164	·163	·161
1·1	·159	·157	·155	·154	·152	·150	·148	·146	·145	·143
1·2	·141	·139	·138	·136	·134	·132	·131	·129	·127	·125
1·3	·124	·122	·120	·119	·117	·115	·113	·111	·110	·108
1·4	·106	·104	·102	·101	·099	·097	·095	·093	·092	·090
1·5	·088	·086	·084	·083	·081	·079	·077	·075	·074	·072
1·6	·070	·068	·066	·065	·063	·061	·059	·057	·056	·054
1·7	·052	·050	·048	·047	·045	·043	·041	·039	·038	·036
1·8	·034	·032	·031	·029	·027	·025	·024	·022	·020	·019
1·9	·017	·015	·014	·012	·010	·008	·007	·005	·003	·002

Estimation of Calcium in Serum (Kramer and Tisdall (22), Tisdall (23), also Clark and Collip (24))

The principle of this method consists in precipitating the calcium as oxalate, and the precipitate after washing and dissolving in sulphuric acid is titrated with permanganate.

Solutions.—1. $\frac{N}{100}$ potassium permanganate solution prepared as required by diluting a $\frac{N}{10}$ permanganate solution,

standardised against an accurate $\frac{N}{10}$ sodium oxalate solution.

2. 4 % ammonium oxalate solution.

3. Ammonia solution, prepared by diluting 2 c.c. of concentrated ammonia (sp. gr. 0·880) to 100 c.c. with distilled water.

Method.—Two c.c. of clear serum or citrated plasma are measured into a 15 c.c. centrifuge tube containing 2 c.c. of water. One c.c. of 4% ammonium oxalate solution is then added and mixed by tapping the tube. The mixture is then allowed to stand for at least thirty minutes, when it is again mixed and centrifuged for five minutes. The supernatant fluid is then removed by decantation and the tube drained for five minutes. Three c.c. of the dilute ammonia are then added from a wash bottle with a fine jet in such a way that the sides of the tube are washed down and the precipitate is well mixed. The tube is then centrifuged and drained as before. The precipitate of calcium oxalate is then dissolved in 2 c.c. of approximately normal sulphuric acid and the tube with its contents is heated in a boiling water bath for a few minutes. While still hot it is titrated with $\frac{N}{100}$ potassium permanganate to a definite pink colour which must persist for at least one minute. During the titration the tube is immersed in a dish of water, the temperature of which is maintained at 80° C. by means of an electric " hot-point " apparatus. A micro-burette graduated in 0·02 c.c. should be used for this titration.

Calculation.—The number of c.c. of permanganate used, multiplied by ten, will equal the number of mg. of calcium per 100 c.c. of serum. The amount of $\frac{N}{100}$ permanganate needed to impart a definite pink colour to 2 c.c. of fluid must be determined and subtracted from the quantity of permanganate used in the final titration.

Estimation of Total Solids in Blood (Myers (16))

The apparatus used in this determination is an ordinary type of weighing bottle with a stopper having a glass loop to which a block of filter paper may be fastened by means of a wire hook. The block of filter paper is suspended and the bottle and the paper dried and weighed. From a small pipette 0·3 to 0·5 g. of the well-mixed oxalated or citrated blood is allowed to flow on the filter paper rapidly, the stopper is quickly inserted and the bottle weighed. The stopper is now tilted and the bottle placed in the drying oven at 105° C. overnight. The bottle is then cooled and again weighed. From the loss in moisture the total solids may readily be calculated.

Normally the total solids amount to 19 to 23%, although in primary and secondary anæmia, severe nephritis, etc., the amount may be decreased to nearly one-half these figures.

COLORIMETRIC URINE ANALYSIS (Folin (25))

Estimation of Ammonia

The principle of this method depends upon the extraction of the ammonia from the urine by means of permutit, a synthetic aluminate powder. The powder must be fine enough to pass through a 60-mesh sieve, but not through an 80-mesh sieve.

Solutions.—1. Standard ammonium sulphate solution, containing 1 mg. of N per 10 c.c. (0·4716 g. of specially purified ammonium sulphate per litre).

2. 10% NaOH.

3. Nessler's solution (see Folin and Wu's methods for blood, p. 382).

Method.—Place about 2 g. of permutit powder in a 100 c.c. flask, add about 5 c.c. of water, and with an Ostwald pipette introduce 2 c.c. of urine. If the ammonia content be low it may be necessary to use more than 2 c.c. Rinse down with

about 5 c.c. of water. Shake gently for five minutes. Rinse powder to bottom of flask with about 30 c.c. of water. Add water once more and decant. Add a little water to powder and 1 c.c. of 10% NaOH. Shake and allow to stand. Dilute to about 75 c.c., add 10 c.c. Nessler's reagent, and make up to the 100 c.c. mark with water.

Preparation of Standard. — Five c.c. of the standard ammonium sulphate solution are placed in a 100 c.c. flask and 1 c.c. of 10% NaOH added. Dilute to about 75 c.c. with water and mix. Ten c.c. of Nessler's reagent are now added, the contents being shaken during the addition. Dilute to the 100 c.c. mark. Compare in the colorimeter with the standard at 20.

Calculation.—Ten, divided by the colorimetric reading, gives the ammonia nitrogen content in milligrams per 2 c.c. The figure thus obtained, if multiplied by 50, will give the answer in milligrams per 100 c.c.

Estimation of Total Nitrogen.

Solutions.—They are the same as those used in the estimation of non-protein nitrogen in blood (see p. 381).

Method.—Dilute 10 c.c. of urine to 100 c.c., mix, and with an Ostwald pipette transfer 1 c.c. of the diluted urine to a large hard glass test tube. Add 2 c.c. of the digestion mixture together with a pebble, to prevent bumping. Heat over a micro-burner until white fumes are emitted, when a watch-glass is placed over the mouth of the tube. When the contents become clear, remove the flame, allow to cool, add water, and rinse into a 200 c.c. flask, using about 125 c.c. of water. Shake the flask, and while shaking add 30 c.c. of Nessler's solution. Dilute to 200 c.c.

Preparation of Standard.—Ten c.c. of the standard ammonium sulphate solution are placed in a 200 c.c. flask, and 2 c.c. of the digestion mixture are added. Dilute to about 150 c.c. and add 30 c.c. of Nessler's solution. Dilute to the

200 c.c. mark. Compare in the colorimeter with the standard at 20.

Calculation.—Two hundred, divided by the colorimetric reading, gives the total nitrogen content in milligrams per c.c. This figure, multiplied by 100, converts the result into milligrams per 100 c.c.

Estimation of Urea

Solutions.—1. Buffer solution. This is the same as for the estimation of blood urea (see p. 382).

2. Urease solution. Wash 3 g. of permutit with 2% acetic acid, then twice with water. Add 5 g. of soya bean meal and 100 c.c. of 15% alcohol. Shake for fifteen minutes. Pour on a large filter and cover with a watch-glass. The filtrate contains the urease, which will keep for four or six weeks in an ice chest, and about a week at room temperature.

3. Nessler's reagent.

Method.—Transfer 1 c.c. of a 1 in 10 diluted urine into a test tube. Add 2 drops of buffer solution and 1 c.c. of the urease solution. Digest in a bath at 40° to 55° C. for five to ten minutes. Rinse into a 200 c.c. flask and dilute to about 150 c.c. Add 20 c.c. of Nessler's reagent and dilute to volume.

Preparation of Standard.—Ten c.c. of the standard ammonium sulphate solution are placed in a 200 c.c. flask and 1 c.c. of urease solution added. Dilute to about 150 c.c. and add 20 c.c. of Nessler's reagent. Make up to the 200 c.c. mark.

Calculation.—Let the colorimetric reading of the unknown be R, then, with the standard at 20, $\frac{200}{R} = NH_3.N +$ urea N in milligrams in 1 c.c. of urine; let this be x mg. Then urea N $= x - NH_3.N$ in 1 c.c. of urine. If the result is to be expressed in milligrams of urea per 100 c.c., then the formula required is $100 \times \frac{15}{7}\left(\frac{200}{R} - NH_3.N\right)$.

Estimation of Uric Acid

Solutions.—1. Acid silver lactate solution.

5 g. of silver lactate.
5 c.c. of lactic acid.
5 c.c. of 10% sodium hydroxide.
75 c.c. of water.

2. Standard uric acid formalin solution. To 0·2 g. of uric acid add 20 c.c. of a 0·5% solution of lithium carbonate. It is advisable to add the lithium carbonate at a temperature of about 60° or 70° C. Stir until all the uric acid has dissolved. Add 10 c.c. of water, 10 c.c. of 40% formalin, and 2 c.c. of 50% acetic acid. Transfer the resulting solution to a 200 c.c. measuring flask and make up to the mark with distilled water. This solution contains 1 mg. of uric acid per c.c. From this stock solution the urine standard can be prepared by making a 1 in 10 dilution.

3. 15% sodium cyanide in $\frac{N}{10}$ NaOH.

4. 20% sodium carbonate solution.

5. Uric acid reagent. Place 100 g. of sodium tungstate in a 2-litre flask and add 50 c.c. of water. Shake. To the solution add 80 c.c. of an 85% phosphoric acid—ordinary phosphoric acid syrup. Close the mouth of the flask with a funnel and two watch-glasses, one small and one large, and boil for two hours. Cool and dilute to 1 litre.

6. 20% lithium sulphate solution.

Method.—In a centrifuge tube containing 3 c.c. of water place 2 c.c. of urine. Add 3 c.c. of the acid silver lactate solution and centrifugate. Decant. Transfer 5 c.c. of the uric acid standard solution into a 100 c.c. flask and add 1 c.c. of the sodium cyanide solution to it and to the tube containing the silver precipitate. When the latter has dissolved, rinse it into a 100 c.c. flask with 50 c.c. of water. Add about 45 c.c. to the standard. To each flask, standard and unknown, add

15 c.c. of the 20% sodium carbonate, 2 c.c. of 20% lithium sulphate, and, while shaking, 3 c.c. of the uric acid reagent. Let stand for four minutes, dilute to volume, mix and compare in the colorimeter with the standard at 20.

Calculation.—Ten, divided by the colorimetric reading, gives the uric acid content in milligrams per 2 c.c. To obtain the answer in milligrams per 100 c.c., multiply the result by 50.

Estimation of Creatinin

Solutions.—1. Standard creatinin solution. 1·61 g. of creatinin zinc chloride is dissolved in 1 litre of $\frac{N}{10}$ HCl (1 c.c. contains 1 mg. of creatinin).

2. Saturated aqueous solution of purified picric acid.

3. 10% NaOH.

Method.—Into two 100 c.c. flasks place respectively—

1 c.c. of urine.	1 c.c. standard creatinin.
20 c.c. picric acid solution.	20 c.c. picric acid solution.
1·5 c.c. 10% NaOH.	1·5 c.c. 10% NaOH.

Stand for ten minutes, dilute to mark, and mix.

Compare in the colorimeter with standard at 20.

Calculation.—Twenty, divided by the colorimetric reading, gives the creatinin content in milligrams per c.c., or, multiplied by 100, in milligrams per 100 c.c.

Estimation of Amino-acid Nitrogen

Solutions.—1. Standard glycine solution. 0·0536 g. of glycine dissolved in 100 c.c. $\frac{N}{10}$ HCl containing 0·2% of benzoic acid. This solution contains 0·1 mg. nitrogen per c.c.

2. Special sodium carbonate solution. Fifty c.c. of a saturated sodium carbonate solution are diluted to a volume

of 500 c.c. The strength of this solution is determined by titrating 20 c.c. of $\frac{N}{10}$ HCl with the carbonate, using methyl red as indicator. The carbonate solution is adjusted until 8·5 c.c. are equivalent to 20 c.c. $\frac{N}{10}$ of HCl.

3. Special acetic acid solution. Add 100 c.c. of 50% acetic acid to 100 c.c. of 5% sodium acetate.

4. A 4% sodium thiosulphate solution.

5. Fresh 0·5% solution of the sodium salt of β-naphthoquinone-sulphonic acid. (See Blood Amino-acid Nitrogen, p. 388.)

Method.—The ammonia is first removed from the urine by means of permutit powder. Dilute 10 c.c. of urine to a volume of 25 c.c. in a 50 c.c. Erlenmeyer flask. Add 2 to 3 g. of permutit powder, shake for five minutes. Decant into another 50 c.c. flask and again add 2 to 3 g. of permutit powder. Shake again for five minutes. Slight turbidity does not interfere with the determination.

Into four 25 c.c. graduated test tubes place—

Five c.c. of the ammonia-free, diluted urine and 1, 2 and 3 c.c. respectively of the glycine standard solution, the latter being the three standards. To the unknown, 1 c.c. of $\frac{N}{10}$ HCl is added. The special sodium carbonate solution is then added, 1 c.c. to the unknown, and 1, 2, 3 c.c. to the standards. Dilute the contents of the four tubes to a volume of 10 c.c.

Dissolve 250 mg. of the β-naphthoquinone salt in 50 c.c. of water, and add 5 c.c. of this solution to each standard and to the unknown.

Mix and stand in the dark overnight. Add 1 c.c. of acetic acid acetate solution and 5 c.c. of 4% $Na_2S_2O_3$ solution to all the tubes. Dilute to 25 c.c.

Calculation.—Let the colorimetric reading of the unknown

be R. Compare with the standard at 20. Then $\frac{20}{R} \times$ strength of standard used = milligrams of amino-acid nitrogen in 10 c.c. urine, and, multiplied by 10 again, gives the content in milligrams per 100 c.c.

Estimation of Phosphates

Solutions. — 1. Standard monopotassium phosphate. 0·351 g. KH_2PO_4 per litre.

2. Molybdic acid solution. 2·5% ammonium molybdate in $5N.H_2SO_4$.

3. 0·1% aminonaphtholsulphonic acid. 0·5 g. of this is dissolved in 500 c.c. of 5% sodium bisulphite to which has been added 5 c.c. of 20% sodium sulphite. If well stoppered this solution keeps for several weeks.

Method.—One c.c. of urine is measured into a 100 c.c. flask, and 5 c.c. of the standard phosphate solution transferred into another 100 c.c. flask. Dilute each to about 70 c.c. and add 10 c.c. of the molybdic acid solution, followed by 10 c.c. of the 0·1% aminonaphtholsulphonic acid solution. Mix after each addition. Make up to the mark and read in the colorimeter after standing four or five minutes.

Calculation.—Five, divided by the colorimetric reading, gives the phosphate content in milligrams of phosphorus per c.c. of urine. This must be multiplied by 100 to give the answer in milligrams per 100 c.c.

Estimation of Proteins in Urine (Hiller, McIntosh and Van Slyke (26))

Solutions.—1. 10% trichloracetic acid solution.

2. 3 % sodium hydroxide solution.

3. 30 % sodium hydroxide solution.

4. Copper sulphate solution containing 20 g. $CuSO_4.5H_2O$ per 100 c.c. solution.

5. Sodium sulphate solution containing 44 g. Na_2SO_4 per 100 c.c. solution. This solution is saturated at 37° C. and must be kept at this temperature to prevent crystallisation.

6. Standard biuret solution containing 0·4 g. of biuret in 150 c.c. solution. This will keep in an ice box for one month.

Total Protein.—Measure 2 c.c. of the urine specimen (previously adjusted to a *p*H of about 7·4) into a centrifuge tube, add an equal volume of trichloracetic acid, mix and centrifuge for five minutes. If the volume of the precipitate be less than 0·2 c.c. or greater than 0·6 c.c., repeat this operation with a greater or smaller volume of urine. Pour off the supernatant fluid. Dissolve the precipitate in about 3 c.c of the 3% sodium hydroxide solution and wash into a tube graduated at 10 c.c. with portions of the 3% sodium hydroxide until the volume is about 9 c.c. Add 0·25 c.c. copper sulphate solution and dilute to 10 c.c. with 3% sodium hydroxide. Mix by shaking, allow to stand ten minutes, centrifuge and compare the colour of the supernatant fluid against that of a standard prepared at the same time.

To prepare the standard, measure 5 c.c. standard biuret solution, containing 13·33 mg. of biuret to 12·3 mg. of protein, into a tube graduated at 10 c.c. Add distilled water to 8 c.c., 1 c.c. of 30% solution hydroxide, 0·25 c.c. copper sulphate solution and dilute to 10 c.c. with water. Mix, allow to stand 10 minutes, centrifuge and compare the colour with that produced by the urine protein, setting the depth of the standard colour at 15 mm.

$$\text{Then } \frac{15}{R} \times \frac{12{\cdot}3}{\text{c.c. urine used}} = \frac{184{\cdot}5}{R \times \text{c.c. urine used}} =$$

g. protein per litre.

Precipitation of Globulin.—To 10 c.c. of urine, adjusted to a *p*H of about 7·4, add 10 c.c. of 44% sodium sulphate solution, mix well and place in an incubator at 37° C. for three hours. Filter.

Albumin.—With the filtrate from the globulin precipitate proceed as for total protein, using four times the volume.

Then $\frac{15}{R} \times \frac{12{\cdot}3 \times 2}{\text{c.c. filtrate used}} = \frac{369}{R \times \text{c.c. filtrate used}} =$ g. albumin per litre.

The globulin is given by the difference between the total protein and the albumin.

CONCLUSIONS

The Value of Blood Analysis

The special importance of each blood constituent has been considered in the earlier chapters of this book, but in order to indicate the general value of the determinations a short summary will be included here.

Nitrogen Distribution.—By means of the Folin and Wu system it is possible to obtain a fairly accurate idea of the distribution of nitrogen throughout the blood filtrate, *i.e.*, the non-protein nitrogen can be divided up between the urea, uric acid, amino-acids, creatinin and similar bodies. If all the well-known non-protein nitrogenous bodies be estimated, and their nitrogen content worked out, it would be expected that the sum of these figures would approximate to the non-protein nitrogen value. This, however, is not so, and there is a relatively large part of the non-protein nitrogen of unknown constitution. Where there is impairment of renal function, the nitrogenous bodies accumulate in the blood, and this unknown fraction of the non-protein nitrogen is also increased. In order to obtain some idea of the variations in this unknown fraction, the French subtract the urea nitrogen from the non-protein nitrogen, and the value thus obtained is called the " non-urea nitrogen " or the "*uræmic moiety.*" As has been indicated in Chapter II., all the nitrogenous bodies form useful indices of the extent of renal damage. The blood uric acid content is raised in the acute

stages of gout, even up to 10 mg. per 100 c.c. and in a large percentage of cases with eczema (Schamberg and Brown (27)). Although renal lesions are by far the commonest cause of nitrogen retention, it has been conclusively proved that acute abdominal conditions, such as appendicitis, intestinal and pyloric obstruction, may be attended with a raised blood urea content.

As de Wesselow (10) emphasises, reliance may be placed in the interpretation of blood figures if the percentage of urea in the urine be estimated simultaneously. This will be diminished in renal disease, but unaltered in acute abdominal conditions. The estimation of amino-acid nitrogen has been advocated in eclampsia and various hepatic lesions. We have never noted any definite or specific alterations in this value.

Sugar.—The blood sugar is of the utmost importance in the diagnosis and treatment of cases of diabetes. (See Chapter III.)

Cholesterol.—The normal cholesterol content of the blood is variously given by different authorities, but that usually accepted is about 150 to 180 mg. per 100 c.c. The cholesterol content is increased in the following conditions : Arteriosclerosis, nephritis, nephrosis, diabetes, obstructive jaundice and cholelithiasis, certain skin diseases, the early stages of malignant disease and in pregnancy. The estimation of blood cholesterol is of the greatest value in cases of chronic nephritis with œdema, and in lipoid nephrosis, as pointed out by Epstein. In the lipæmia of diabetes mellitus, the cholesterol content may rise to 600 to 800 mg. per 100 c.c. In very extreme cases figures of over 1,410 mg. per 100 c.c. have been recorded. When gall-stones are present there is usually hypercholesterolæmia. Campbell (28) suggests that this is only present if there be accompanying obstructive jaundice, and that the determination of blood cholesterol is of no value in the diagnosis of gall-stones. Hypercholesterolæmia has been described in the early stages of

malignant disease, and is well known in pregnancy. The cholesterol content of the blood is reduced in the anæmias, and it has been thought that these conditions are benefited by the administration of cholesterol. A series of excellent reviews on the value of blood cholesterol estimations have been published by Gorham and Myers (29), Grigaut (30), Chauffard, Laroche and Grigaut (31).

Phosphates.—The phosphate content of the serum is increased in chronic nephritis with nitrogen retention from a normal of about 3 mg. to 20 to 30 mg. per 100 c.c. De Wesselow lays great stress upon this point, and his work has already been referred to. In rickets the phosphate content is usually decreased.

Calcium.—The normal figures are said to be between 9 and 11 mg. per 100 c.c. The calcium is not all present in ordinary solution, since it has been conclusively proved that 30 to 40% is indiffusible, and may be in combination with some constituent such as the proteins. The content is usually decreased towards the end of pregnancy. De Wesselow showed that a reduction of the calcium content frequently occurred in the terminal stages of chronic nephritis, and that as the inorganic phosphate content rose so the calcium decreased. Figures as low as 5 to 6 mg. per 100 c.c. were obtained.

In 1925 Collip (32) obtained a potent extract containing the active principle of the parathyroid glands which can be purchased under the name of "parathormone." This extract was prepared in the following manner: Glands were removed from oxen, the fat etc. separated, and the glands frozen at once. They were kept in an ice-box at — 4° C., and when required for use a few glands were placed in a large Pyrex test tube and covered with an equal volume of 5% HCl. The test tube was then heated in a boiling-water bath for one hour, the glands being broken up after a few minutes with a glass rod. The fat on the surface was then removed, the extract chilled to room temperature and made alkaline to *p*H 8 by NaOH. HCl was added slowly till a maximum pre-

cipitation occurred. The precipitate was removed either by filtering or centrifugalisation and re-dissolved in weak alkali. A second iso-electric precipitation was carried out and the filtrates or supernatant fluids combined, this preparation representing an aqueous solution of the active principle. The unit of potency was defined as 1/100 of the amount of extract which will produce an average increase of 5 mg. in the blood serum calcium of normal dogs of approximately 20 kilos weight over a period of fifteen hours (33). This work has been repeated by Davies, Dickens and Dodds (34), who throw some doubt upon the specificity of the hormone. They also evolved a method for the extraction of material on similar lines to that for the preparation of insulin by the acetone picric acid process.

Clinical Applications.—Parathyroid extract is used mainly in controlling the tetany following thyroparathyroidectomy. It has been shown that in this condition the calcium content of the blood is decreased, and that administration of the hormone produces an increase in the blood calcium, so that by this means it can be restored to normal. Judging from the publications which have appeared up to the present, no definite dosage of the hormone can be laid down, and the administration must be controlled by frequent blood calcium estimations. Probably 5 units of the extract per kilogram of body-weight for the first twenty-four hours would be sufficient to raise the blood calcium to normal limits. Following this the dose can be reduced to a minimum to maintain the blood calcium normal.

Hoag and Rivkin (35) also found the treatment of infantile tetany with a parathyroid extract to be very effective. Their experiments show that injections of 5 units of the extract per kilogram of body-weight for a period of about twenty-four to thirty-six hours produce a rise of about 1 mg. of the serum calcium per 100 c.c. The symptoms improve rapidly and the blood calcium returns to normal within twenty-four to forty-eight hours. These observers, however,

emphasise the fact that great caution in the administration of the extract is required, and point out the necessity for repeated blood calcium estimations.

The hormone has also been employed by Gordon, Roark and Lewis (36) in the treatment of tuberculosis, where it was found that daily injections of from 10–20 units produced a rise of 2 or 3 mg. of calcium above the normal level of 9–11 mg. per 100 c.c. of blood within twelve hours of administration, so that it was possible to maintain by means of this dosage a calcium level of between 12 and 14 mg. per 100 c.c. It was found that, although some of the symptoms may be benefited, the general course of the disease remains unaffected under this treatment. Pulmonary hæmorrhage, however, was always relieved by injections of the hormone in doses of from 30–40 units during a period of thirty-six hours, followed by a dose of 10 units every second day, or in some cases less frequently, for two or three weeks. It is obvious, however, that one cannot hope to control hemoptysis due to the erosion of a large blood vessel. It would appear that this form of treatment is mainly of theoretical interest.

Hunter and Aub (37) have shown that the excretion of calcium from the bones after administration of Collip's parathyroid extract is also accompanied by an excretion of lead in patients suffering from lead poisoning, and they have therefore employed the extract in treating cases of lead poisoning with some success. The dosage was regulated very largely according to the level of the blood calcium. It was generally found that by giving an average dose of 55 units daily the blood calcium could be increased from an average figure of 8·8 to about 13 mg. or more within eight or nine days. The doses were given three times a day and the daily dosage could be increased to 100 units by adding with caution 10 units daily. The level of the blood calcium was kept above normal for periods of time varying from three days to three weeks. In some cases a mild hypercalcæmia was pro-

duced and maintained for six days without ill effect. These observers also emphasise the necessity for adequate laboratory control, and point out the risks attendant upon the treatment, advising its use only in certain selected cases.

In rickets the calcium is often unaffected, whilst the phosphate content is reduced. It is possible, however, to produce in animals experimental rickets associated with a very low calcium content. Schwarz and Levin (38) have recorded a low blood calcium content in acne vulgaris, eczema, gummatosis and folliculitis. Mark (39) found considerably increased blood calcium figures in a series of cases of arthritis deformans. The calcium content is also said to be increased in otosclerosis.

Total Solids and Hæmoglobin.—The value of these determinations lies in the fact that they indicate whenever there is concentration of the blood due to dehydration of the patient. Thus, in diabetic coma, the degree of dehydration may be estimated by either of these methods. If the solid content or hæmoglobin is found to be considerably increased, a condition similar to the " sticky " blood described on p. 124 will be found, and the appropriate treatment may be given. A prolonged experience with blood analysis indicates that these tests should be done wherever possible, as nitrogen retention may be partly due to concentration of the blood as shown by an increase in the above values.

REFERENCES

1. Garrod.
" *Observations on Certain Pathological Conditions of the Blood and Urine in Gout, Rheumatism, and Bright's Disease.*" *Med. Chir. Trans.*, 1848, xxxi., 83–97.
2. Schmidt.
" *Charakteristik der Epidemischen Cholera,*" 1850, 167. Leipsig und Mitau.
3. Bang.
" *Ueber die Micromethode der Blutzuckerbestimmung.*" *Biochem. Zeit.*, 1913, lvii., 300–312.
Die Blutzucker, 1913, 162. Wiesbaden.

4. Folin and Wu.
Various papers in *Journ. of Biol. Chem. Journ. Biol. Chem.*, 1919, xxxviii., 81–110 ; 1920, xli., 367–377 ; 1920–21, xlv., 449–460 ; 1922, li., 377–391 ; 1922, liv., 153–170.
Folin.
"*Laboratory Manual of Biological Chemistry*," 1925, 227–305. D. Appleton & Co., New York.
5. Tolstoi.
Journ. Biol. Chem., 1924, lx., 69.
6. Folin and Doisy.
Journ. Biol. Chem., 1916, xxvii., 349.
7. Benedict.
Journ. Biol. Chem., 1929, lxxxii., 1.
8. Van Slyke.
Journ. Biol. Chem., 1923, lviii., 523.
9. Myers and Wardell.
Journ. Biol. Chem., 1918, xxxvi., 147.
10. de Wesselow.
"*Chemistry of the Blood in Clinical Medicine*," 1924, 244. Ernest Benn, Ltd., London.
11. Bell and Doisy.
Journ. Biol. Chem., 1920, xliv., 55.
12. Lehman.
Journ. Biol. Chem., 1921, xlviii., 293.
13. Dennis and von Meysenbug.
Journ. Biol. Chem., 1922, lii., 1.
14. Tolstoi.
Journ. Biol. Chem., 1923, lv., 157.
15. Hawk and Bergeim.
"*Practical Physiological Chemistry*, 9th edn., 1926. Churchill, London.
16. Myers.
"*Practical Chemical Analysis of Blood*," 1924. Kimpton, London.
17. Bernheim.
Journ. Amer. Med. Assocn., 1924, lxxxii., 291.
18. Marshall.
Journ. Biol. Chem., 1913, xv., 487.
19. Van Slyke and Cullen.
Journ. Biol. Chem., 1914, xix., 211.
20. MacLean.
"*Modern Methods in the Diagnosis and Treatment of Renal Disease*," 3rd Edn., 1927. Constable, London.
21. Hagedorn and Jensen.
Biochem. Zeit., 1923, cxxxv., 46.
22. Kramer and Tisdall.
Journ. Biol. Chem., 1921, xlvii., 475.

23. Tisdall.
Journ. Biol. Chem., 1923, lvi., 439.
24. Clark and Collip.
Journ. Biol. Chem., 1925, lxiii., 461.
25. Folin.
"*Laboratory Manual of Biological Chemistry*," 1925, 117–170. D. Appleton & Co., New York.
26. Hiller, McIntosh and Van Slyke.
Journ. Clin. Invest., 1927, iv., 235.
27. Schamberg and Brown.
Amer. Journ. Dermat. and Syphil., 1923, viii., 801.
28. Campbell.
Quart. Journ. Med., 1924, xviii., 123.
29. Gorham and Myers.
Arch. Int. Med., 1917, xx., 599.
30. Grigaut.
Biol. Medicale, 1913, xl., 89.
31. Chauffard, Laroche and Grigaut.
L'Obstetrique, 1911, n.s., iv., 481.
32. Collip.
Journ. Biol. Chem., 1925, lxiii., 395.
Collip and Clark.
Journ. Biol. Chem., 1925, lxiv., 485.
33. Collip and Clark.
Journ. Biol. Chem., 1925, lxvi., 133.
34. Davies, Dickens and Dodds.
Biochem. Journ., 1926, xx., 695.
35. Hoag and Rivkin.
Journ. Amer. Med. Assocn., 1926, lxxxvi., 1343.
36. Gordon, Roark and Lewis.
Journ. Amer. Med. Assocn., 1926, lxxxvi., 1683.
37. Hunter and Aub.
Quart. Journ. Med., 1927, xx., 123.
38. Schwarz and Levin.
Arch. Dermat. and Syphil., 1924, x., 544.
39. Mark.
Journ. Metabol. Res., 1923, iv., 135.

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